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Membrane Lipid Biosynthesis in *Acholeplasma laidlawii* B: Incorporation of Exogenous Fatty Acids into Membrane Glyco- and Phospholipids by Growing Cells

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The extent of incorporation of a wide variety of exogenous saturated, unsaturated, branched-chain, and cyclopropane fatty acids into the membrane lipids of *Acholeplasma laidlawii* B was systematically studied. Within each fatty acid class the extent of incorporation generally increased markedly with increasing chain length, reached a maximum, and then declined progressively but less sharply with further increases above that chain length giving maximal direct incorporation. Certain shorter-chain members of each fatty acid class underwent complete or partial conversion to longer-chain homologues before utilization for complex lipid biosynthesis. The degree and extent of chain elongation and direct incorporation and the characteristic dependence of each of these processes on fatty acid chain length and structure correlated well with the physical properties (melting temperatures) of the exogenous fatty acids. The in vivo specificity of the enzyme systems responsible for the incorporation of exogenous fatty acids was such that the fluidity and physical state of the membrane lipids were maintained within a definite, albeit a relatively wide, range. We also observed that the neutral glycolipids typically have similar fatty acid compositions, which are somewhat different from those of the major phosphatides, which also exhibit similar fatty acid spectra. The phosphorylated glycolipid glycerophosphoryldiglucosyl diglyceride, however, always maintained a unique fatty acid composition quite different from that of the diglucosyl diglyceride from which it is presumably derived. These characteristic differences in fatty acid composition appear to function to minimize differences in phase transition temperatures, thus producing a more physicochemically homogeneous mixture of membrane lipids than would result from a nonspecific incorporation of fatty acids.

The procaryotic microorganism *Acholeplasma laidlawii* B possesses a number of characteristics that make it particularly suitable for in vivo investigations of the specificity, regulation, and interrelationships of the enzyme systems responsible for the formation of membrane glyco- and phospholipids. These characteristics include the absence of a cell wall or internal membrane system, the genetic and metabolic simplicity of this organism, and the degree to which the fatty acid composition and sterol content of the membrane lipids may be altered (for reviews, see 18 and 26). The absence of a lipid-containing cell wall or internal membrane makes possible the straightforward isolation of pure plasma membranes, which contain essentially all of the membrane lipid and nearly a third of the total cell protein (18, 26). The small genome size of this organism is manifested in its limited ability to synthesize and degrade fatty acids and complex membrane lipids. Although *A. laidlawii* B is capable of the de novo formation of certain even-numbered saturated fatty acids, cells grown in a lipid-free medium cannot synthesize unsaturated, branched-chain, or cyclopropane fatty acids (12, 25, 32, 33). This organism also lacks the capability for the α, β, or ω oxidation of endogenous or exogenous fatty acids (44). Similarly, although *A. laidlawii* B can synthesize all of its major membrane glyco- and phospholipids (for review, see 44), these lipids are not degraded or otherwise altered after their formation, at least under normal growth conditions (13, 20). The relatively uncomplicated metabolic pathways leading to the formation of the major membrane lipids and the absence of metabolic turnover of either intact glyco-

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and phospholipid molecules or their fatty acid, glycerol, or polar headgroup components facilitates the study of fatty acid and membrane lipid metabolism in living cells. In addition, the ability to manipulate the fatty acid and sterol composition makes it possible to investigate directly the response of the various enzyme systems to alterations in the composition, fluidity, and physical state of the membrane lipids. *A. laidlawii* B has already been demonstrated to be an excellent system in which to study the role of lipids in biological membranes (for reviews, see 18 and 26).

The present study is concerned with the incorporation of exogenous fatty acid into the membrane glyco- and phospholipids. We reported earlier that *A. laidlawii* B can extensively incorporate certain exogenous saturated, unsaturated, and branched-chain fatty acids into the membrane polar lipids (19, 27, 31). Although the variety of different fatty acids that can be utilized by this organism is large, the extent of incorporation can vary greatly depending on the chemical nature and chain length of the exogenous fatty acid (19, 27, 31). However, the results of a detailed and systematic study of the uptake and biosynthetic incorporation of exogenous fatty acids have not been reported. In this paper we present data on the extent of incorporation of a variety of saturated, unsaturated, branched-chain, and cyclopropane fatty acids into the total membrane lipids of *A. laidlawii* B. The object of this study was to determine the relationship between the structure, and thus the chemical and physical properties, of an exogenous fatty acid and its suitability for uptake and incorporation into the membrane lipids of this organism. Such a study also serves to delineate the in vivo specificity of the enzyme system responsible for the biosynthetic utilization of exogenous fatty acids. In addition, the fatty acid compositions of the individual glyco- and phospholipids from cells grown without fatty acid supplementation and in the presence of various exogenous fatty acids are determined and compared. The object of these latter experiments was to determine if particular membrane lipids maintain characteristic fatty acid spectra and, if so, to see if this phenomenon might reflect their metabolic origin and/or functional role in the membrane.

MATERIALS AND METHODS

Organism. The organism used throughout these studies was *A. laidlawii* (formerly *Mycoplasma laidlawii*), strain B, originally obtained from D. G. ff. Edward (Wellcome Research Laboratories, Beckenham, Kent, England).

Conditions of growth. Cells were grown without shaking at 35°C in a lipid-poor growth medium, the preparation of which has been described previously (21). The total free fatty acid content of this medium, before and after addition of the fatty acid-poor bovine serum albumin used to detoxify exogenous fatty acid, is presented in Table 1. When serum albumin is present in the growth medium, it is the major source of residual fatty acid, contributing nearly three-fourths of the total free fatty acid present. However, the lipid-poor growth medium and albumin together contribute less than 10% of the total free fatty acid present after exogenous fatty acids have been added to the growth medium, as described below. The fatty acid content of *A. laidlawii* B cells grown on this lipid-poor medium, with or without exogenous fatty acid supplementation, is described in detail in reference 41. Considerable quantities of esterified fatty acid are also present in the lipid-poor tryptose medium, but this organism is unable to utilize fatty acids in this form.

Ethanolic solutions (10 mg/ml) of the exogenous fatty acid to be tested were added to the growth medium before inoculation to give a final fatty acid concentration of 0.12 mM; the final concentration of ethanol in the growth medium was 0.5% (by volume) or less. The incorporation of all exogenous fatty acids was found to increase proportionately with increases in fatty acid concentration up to some limiting value characteristic of each individual fatty acid; the exogenous fatty acid concentration of 0.12 mM used in this study results in the maximum incorporation of all but a few of the very shortest-chain fatty acids tested. Cells were harvested by centrifugation (13,000 × *g*, 15 min) in the late log or early stationary phase of growth 16 to 20 h after a 1% inoculation with mid-log-phase cells. Cell growth was monitored by measuring the absorbancy at 450 nm.

Extraction of membrane lipids. The total mem-

TABLE 1. *Content of residual free fatty acid remaining in the lipid-poor tryptose growth medium before and after the addition of fatty acid-poor bovine serum albumin*

Fatty acid present[a]	Fatty acid content (μM)	
	Without albumin	With albumin
12:0	0.12	0.12
14:0	0.15	0.15
16:0	0.90	2.42
18:0	0.65	4.58
18:1*c*△9	1.14	2.60
18:2*c*, *c*△9, 12	0.44	2.51

[a] The number immediately preceding the colon indicates the total number of carbon atoms and the number after the colon denotes the number of double bonds, if any, present in the fatty acid. The letter *c* indicates the *cis*-configuration of the double bonds. The number after the symbol △ in the unsaturated fatty acid series indicates the position(s) of the double bond(s) within the fatty acid hydrocarbon chain.

brane lipids were quantitatively extracted from cells by a modification of the method of Bligh and Dyer (2). The cell pellet from 1 liter of culture medium was triturated with 40 ml of water, mixed with 150 ml of methanol-chloroform (2:1, vol/vol), and centrifuged (650 × *g*, 15 min). The supernatant was removed and retained, and the cell pellet was extracted a second time. The combined one-phase chloroform-methanol-water solution was mixed well with 200 ml of chloroform, 200 ml of water, and 400 ml of chloroform in that order. Subsequent centrifugation (650 × *g*, 15 min) resulted in a clean separation into two phases; the upper methanol-water phase and the interphase contained the cellular proteins and other nonlipid material, whereas the lower chloroform phase contained the membrane lipid. The upper phase and the interphase material were then removed by aspiration. After reducing the volume by rotatory vacuum evaporation, the chloroform solution was passed through a chromatographic column prepared from 5 g of silicic acid in chloroform, and the column was washed once with 100 ml of methanol. The resultant eluant contained the total membrane lipid essentially free of nonlipid contaminants.

Fractionation and purification of the membrane polar lipids. The individual glyco- and phospholipids were separated and purified by a combination of column chromatography and thin-layer chromatography. The total membrane lipid was first separated into three fractions by Unisil silicic acid column chromatography by a modification of the method of Vorbeck and Marinetti (47). About 15 g of Unisil, previously washed with methanol and activated overnight by heating at 110°C, was packed into a chromatographic column as a hexane slurry and then successively washed with 75 ml of hexane, 60 ml of diethyl ether, and 150 ml of chloroform. The total membrane lipid from cells grown in 1 liter of culture medium was applied to the Unisil column as a 10-ml chloroform solution, and the flask containing the total lipid solution was washed with an additional 10 ml of chloroform, which was also applied to the column. The column was then washed with 50 ml of chloroform and 50 ml of chloroform-acetate (6:1, vol/vol) to quantitatively elute the neutral lipids. This first fraction was then discarded. The Unisil column was subsequently washed with 250 ml of acetone to quantitatively elute the neutral glycolipids that constitute fraction II. Finally, the column was washed with 100 ml of methanol to elute the phosphorus-containing lipids that make up fraction III. Fractions II and III were then separated into their individual components by thin-layer chromatography on glass plates (5 by 20 cm) coated to a thickness of 500 μm with Silica Gel H and developed in solvent systems of chloroform-methanol-glacial acetic acid-water (80:20:4:2, vol/vol) and chloroform-methanol-water-7 N ammonium hydroxide (70:30:3.5:2, vol/vol), respectively. The two components resolved from fraction II and the three components resolved in fraction III were identified by comparing their chemical composition and R_f values with those previously reported by Shaw et al. (36, 37). The position of each lipid on the thin-layer chromatographic plate was visualized by a brief exposure to iodine vapor, after which the Silica Gel H was scraped from the appropriate areas of the chromatoplate and the individual lipids were eluted with 10 ml of chloroform-methanol (1:9, vol/vol).

Analysis of fatty acids by gas-liquid chromatography (GLC). Methyl esters of fatty acids were prepared from complex polar lipids by transesterification by heating with acidified anhydrous methanol according to the method of Gander et al. (11). The lipids were dried in a screw-capped test tube (15 by 1.5 cm) with a Teflon liner. To the test tube were added 10 ml of methanol and several drops of concentrated sulfuric acid, and the tube was heated at 65°C for 2 h. After cooling, the contents of the tube were transferred to a 125-ml separatory funnel and mixed with 20 ml of water, and the methyl esters were extracted twice with 10 ml of hexane. The extracted hexane solution was dried with anhydrous sodium sulfate. A smaller-scale extraction method was also successfully used, especially when the volume of the culture medium was less than 250 ml. The hexane extracts were injected into the gas-liquid chromatograph after concentration by evaporation.

The gas-liquid chromatograph used for the qualitative and quantitative analysis of fatty acid esters was a Hewlett-Packard model 5711A (San Diego, Calif.) equipped with dual columns, hydrogen flame ionization detectors, a model 7128A dual strip chart recorder, and a model 3370B electronic integrator. The chromatographic column (stainless steel, 0.5 inch by 6 feet [ca. 1.3 by 183 cm]) was packed with 10% diethylene glycol succinate on Anachrom ABS, 70/80 mesh, support (Analabs, North Haven, Conn.). Chromatographic conditions were as follows: column temperature, initial – 80 to 120°C, final – 210°C; temperature raise, 4 or 8°C/min; carrier gas, helium; flow rate, 60 to 100 ml/min; range of sensitivity, 100 or 1,000. Fatty acids were identified on the basis of retention times as compared with known standards. The proportion of each fatty acid present in a sample was calculated from the area under each peak, as determined by the electronic integrator.

Analysis of fatty acid relative specific activity with the radioactivity monitoring-GLC system. Since *A. laidlawii* B is capable of the de novo biosynthesis of even-numbered, straight-chain saturated fatty acids, the extent of the direct and total incorporation of these exogenous fatty acids cannot be accurately determined by GLC alone, in contrast to the other exogenous fatty acid classes examined. The extents of incorporation of even-numbered, straight-chain saturates were therefore measured with the use of radioactive exogenous fatty acids and an on-line radioactivity monitoring system, model S190 (Nuclear-Chicago, Des Plains, Ill.). The radioactivity monitoring-GLC system provides a simultaneous analysis of the mass and radioactivity of each fatty acid emerging from the GLC. The proportion of each fatty acid derived from endogenous and exogenous sources can be calculated from

the relative specific activity of that fatty acid after proper calibration of the system.

Materials. The nonradioactive, straight-chain saturated and unsaturated fatty acids were obtained from Nu-Chek-Prep, Inc. (Elysian, Minn.), and the branched-chain and cyclopropane fatty acids were obtained from Analabs, Inc. (North Haven, Conn.). All nonradioactive fatty acids had a purity of greater than 99%. Sodium-[1-^{14}C]butyrate and 1-^{14}C-labeled dodecanoic, tetradecanoic, and hexadecanoic acids were purchased from New England Nuclear (Boston, Mass.). Sodium [1-^{14}C]hexanoate and 1-^{14}C-labeled octanoic and decanoic acids were from International Chemical and Nuclear Corp. (Irvine, Calif.). All radioactive fatty acids had a radiopurity of greater than 99%. Unisil-activated silicic acid, 200 to 325 mesh, was obtained from Clarkson Chemical Co., Inc. (Williamsport, Pa.), and Silica Gel H (type 60) was purchased from Brinkmann Instruments (Canada) Ltd. (Rexdale, Ontario). All organic solvents were of reagent grade and were redistilled before use.

RESULTS

In the following sections the extent of direct incorporation is defined as the amount of a particular fatty acid incorporated into the membrane lipids in a chemically unaltered form, i.e., without chain elongation. The extent of total incorporation is considered to be the total quantity of an exogenous fatty acid and its elongation products (if any), which are utilized for membrane lipid biosynthesis. A detailed in vivo characterization of the fatty acid elongation system itself will be presented elsewhere (Y. Saito, J. R. Silvius, and R. N. McElhaney, J. Bacteriol., in press).

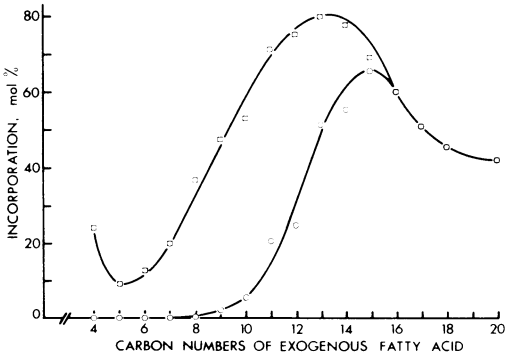

FIG. 1. *Effect of fatty acid chain length on the extent of the direct* (○) *and total* (□) *incorporation of exogenous straight-chain saturated fatty acids into the total membrane lipids of A. laidlawii B. The extents of direct and total incorporation, expressed here as the mole percent of the total esterified fatty acid, were determined by GLC (odd-numbered saturates) or by radioactivity monitoring-GLC (even-numbered saturates) as described in the text; each value presented is the average of at least three independent experiments.*

Incorporation of straight-chain saturated fatty acids. The extent of direct and total incorporation of exogenous straight-chain saturated fatty acids into the total membrane lipids of *A. laidlawii* B is plotted as a function of fatty acid chain length in Fig. 1. Considering first only direct incorporation, one notes that the short-chain fatty acids (C_4 to C_8) are not incorporated as such into the membrane lipids. Further increases in the chain length of the exogenous saturated fatty acids result in a marked and progressive increase in the extent of direct incorporation, with maximum incorporation (about 67 mol%) being obtained with the C_{15} acid. Increasing the chain length still further results in a progressive but less marked decrease in the extent of direct incorporation.

When the extent of total incorporation of the various exogenous saturated fatty acids is plotted as a function of fatty acid chain length, a somewhat different pattern emerges. The curve of incorporation versus carbon number is shifted toward the shorter-chain fatty acids, and the maximum extent of total incorporation obtained (about 80 mol%) is greater than the extent of direct incorporation. However, although the curve is broader, the extent of total incorporation still increases with increases in the chain length, reaches a maximum incorporation, but at a shorter chain length (C_{13}), and then declines with further increases in carbon number. The magnitude of the differences noted between total and direct incorporation is greatest for the short-chain saturated fatty acids (C_4 to C_8), since these compounds undergo quantitative conversion primarily to C_{13} and C_{15} or C_{14} and C_{16} fatty acids, and tends to diminish as one approaches the C_{15} acid, which undergoes only a small amount of chain elongation. The extents of direct and total incorporation of the longer-chain saturates (C_{16} to C_{20}) are the same, since no chain elongation of these fatty acids occurs.

It is of interest to note that the total incorporation of the C_4 saturate is greater than that of the next three members of the series, with minimal total incorporation being noted for the C_5 acid. We will return to this point in a later section.

Incorporation of exogenous isobranched fatty acids. The extent of direct and total incorporation of exogenous isobranched saturated fatty acids is plotted as a function of fatty acid chain length in Fig. 2. In general, these curves resemble those obtained with the straight-chain saturated fatty acids, although several

quantitative differences can be noted. The extent of direct incorporation increases markedly and progressively as the chain length of the exogenous isobranched acids is increased from C_{10} to C_{16}; isobranched fatty acids containing eight carbon atoms or less are not incorporated as such into the membrane lipids. Further increases in carbon number result in a progressive but less marked decrease in the extent of direct incorporation. The chain length for maximum incorporation in the isobranched series is greater than that observed with the straight-chain saturates (C_{16} versus C_{15}), and the extent of direct incorporation at the optimum chain length is also greater (about 85 versus 67 mol%). The curve of total incorporation of the isobranched fatty acids as a function of fatty acid chain length is also shifted toward the shorter-chain fatty acids, but in contrast to the straight-chain saturated fatty acid series, the maximum extents of total and direct incorporation are both obtained with the same fatty acid (C_{16}) and are equal. Short-chain isobranched fatty acids (C_4 to C_8) undergo quantitative conversion primarily to C_{14} and C_{16} isobranched acids, and the extent of chain elongation decreases progressively as one proceeds from C_{10} to C_{14}. Isobranched fatty acids containing 16 carbons or more are not substrates for the chain elongation system, so that their extents of direct and total incorporation are equal. It should be noted that the introduction of a methyl isobranch shifts the entire curves of both direct and total incorporation toward higher carbon numbers. Thus, isobranching tends to decrease the extent of direct incorporation and increase the extent of chain elongation of fatty acids having less than the optimum number of carbon atoms for maximum incorporation and to increase the extent of direct (and total) incorporation of fatty acids having a greater than optimum chain length relative to the straight-chain saturated fatty acid series.

One can again note that the total incorporation of the C_4 fatty acid is greater than that of the next three members of the isobranched series tested, with minimum total incorporation being obtained with the C_6 acid.

Incorporation of exogenous anteisobranched fatty acids. The extent of direct and total incorporation of exogenous anteisobranched fatty acids is plotted as a function of fatty acid chain length in Fig. 3. These curves closely resemble those of the isobranched fatty acids in all respects, except that both the direct and total incorporation curves are shifted even further toward longer chain lengths. The fatty acid giving maximum direct and total incorporation is C_{17} in the anteisobranched series, as compared to C_{15} and C_{16} in the straight-chain and isobranched saturated fatty acid series, respectively. The extent of direct incorporation increases markedly as one proceeds from the C_{11} to the C_{17} fatty acid; the shorter-chain members of this series (C_5 to C_9) are again not incorporated as such into the membrane lipids. Increases in chain length above C_{17} result in a progressive but less marked decrease in the extent of direct incorporation. The maximum extent of direct incorporation (roughly 83 mol%) is similar to that noted for the iso-

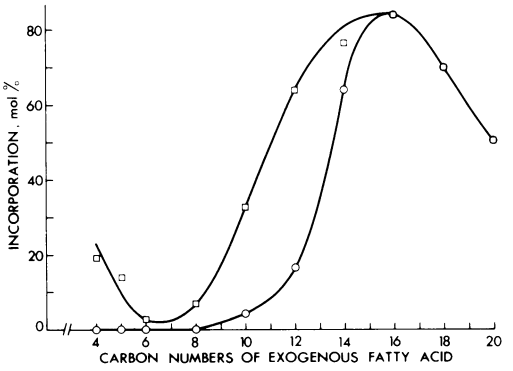

FIG. 2. *Effect of fatty acid chain length on the extent of the direct and total incorporation of exogenous methyl-isobranched fatty acids into the total membrane lipids of A. laidlawii B. The extents of direct (○) and total (□) incorporation, expressed as the mole percent of the total esterified fatty acid, were determined by GLC and represent the averages of at least three independent experiments.*

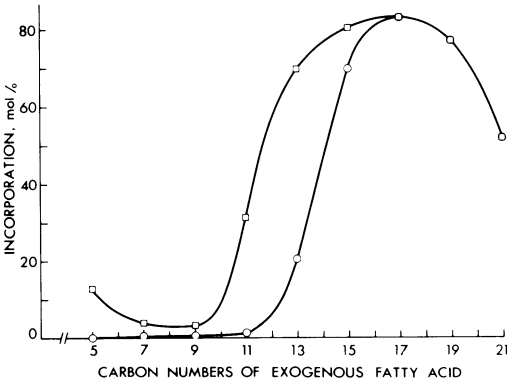

FIG. 3. *Effect of fatty acid chain length on the extent of the direct and total incorporation of exogenous methyl-anteisobranched fatty acids into the total membrane lipids of A. laidlawii B. The extents of direct (○) and total (□) incorporation, expressed as the mole percent of the total esterified fatty acid, were determined by GLC and represent the averages of at least three independent experiments.*

branched fatty acid series but somewhat greater than that obtained with the straight-chain saturates. The curve of total incorporation versus fatty acid chain length is again shifted in the direction of the lower-carbon-number fatty acids. Anteisobranched fatty acids containing 5 to 11 carbon atoms are nearly quantitatively converted primarily to their C_{15} and C_{17} homologues, and the extent of chain elongation decreases as one proceeds toward the C_{15} fatty acid. Anteisobranched fatty acids containing 17 to 21 carbon atoms do not undergo chain elongation before their biosynthetic utilization by this organism. A chain length minimum is again noted here at the C_9 fatty acid, with the extent of total incorporation increasing as one proceeds either toward the shorter- or longer-chain-length members of this series.

Incorporation of exogenous unsaturated and cyclopropane fatty acids. The extent of direct and total incorporation of various exogenous monounsaturated, polyunsaturated, and cyclopropane fatty acids are presented in Table 2, along with similar data for selected straight-chain saturated fatty acids for purposes of comparison. Unfortunately, only a relatively small number of unsaturated and cyclopropane fatty acids are commercially available, so that the range of chain lengths that could be examined in each series is much restricted in comparison to the straight- and branched-chain saturated fatty acid series, particularly with regard to the shorter-chain fatty acids.

The general pattern of direct incorporation observed in the *cis*-monounsaturated fatty acid series is similar to that already observed with the straight- and branched-chain fatty acids. The extent of direct incorporation again increases markedly as the carbon number increases, the maximum direct incorporation being obtained with the 18-carbon acid; the chain length optimum for direct incorporation would appear to lie between 18 and 19 carbon atoms. Further increases in chain length result in a progressive but somewhat less marked decrease in the extent of direct incorporation. The entire curve of direct incorporation versus fatty acid chain length is shifted further in the direction of the longer-chain fatty acids in the *cis*-monounsaturates than in either the straight-chain saturated or branched-chain fatty acid series. The extent of direct incorporation observed at the optimum chain length (about 69 mol%) is slightly greater than that observed with the straight-chain saturates but less than that obtained in the iso- and anteiso-branched fatty acid series. In contract to the straight- and branched-chain saturated fatty acids, the extent of total incorporation of *cis*-monounsaturates is relatively constant from C_{14} to C_{18} instead of increasing as one proceeds toward the chain length of maximum direct (and total) incorporation. The 14-carbon fatty acid undergoes extensive chain elongation primarily to its 16- and 18-carbon homologues, but the extent of elongation decreases rapidly as the chain length increases. *cis*-Monounsaturates containing 20 or more carbon atoms do not undergo chain elongation before their utilization for membrane glyco- and phospholipid biosynthesis.

Although only three members of the *trans*-monounsaturated fatty acid series were tested, their extents of direct and total incorporation can be compared with those of members in the other series examined which have similar carbon numbers. The extents of direct incorporation of the C_{14} and C_{16} *trans*-monounsaturates are about twice those of the corresponding *cis*-isomers. The chain length giving optimum di-

TABLE 2. *Extent of direct and total incorporation of exogenous unsaturated and cyclopropane fatty acids into the total membrane lipids of A. laidlawii B*

Exogenous fatty acid[a]	Incorporation (mol%)	
	Direct	Total
cis-Monounsaturated		
14:1*c*△9	28.6[b]	72.6[b]
16:1*c*△9	36.2	69.9
18:1*c*△9	68.9	71.7
20:1*c*△11	58.3	58.3
22:1*c*△13	36.6	36.6
24:1*c*△15	15.9	15.9
trans-Monounsaturated		
14:1*t*△9	41.6	80.8
16:1*t*△9	78.8	79.7
18:1*t*△9	82.4	82.4
cis-Polyunsaturated		
18:2*c*,*c*,*c*△9,12	47.6	57.3
18:3*c*,*c*,*c*△9,12,15	36.4	56.3
20:4*c*,*c*,*c*,*c*△5,8,11,14	31.7	31.7
Cyclopropane		
19:0*cp*,*c*△9	57.8	57.8
19:0*cp*,*t*△9	67.4	67.4
Straight-chain saturated		
14:0	55.4	77.9
16:0	60.4	60.4
18:0	45.2	45.2
20:0	43.0	43.0

[a] The presence of a cyclopropane ring is indicated by *cp*; *c* and *t* indicate the *cis*- and *trans*-configurations, respectively, of the double bonds or cyclopropane ring systems. The number after the symbol △ in the unsaturated and cyclopropane fatty acid series indicates the position(s) of the double bond(s) or ring systems within the fatty acid hydrocarbon chain.

[b] These values are the average of at least triplicate experiments.

rect incorporation would appear to lie between 16 and 18 carbon atoms, somewhat less than observed in the *cis*-monounsaturated series. Again, as noted for the *cis*-monounsaturates only, the extent of total incorporation is nearly constant from C_{14} through C_{18}. The degree of chain elongation of the C_{14} and C_{16} *trans*-monounsaturates is much less than that observed for the corresponding *cis*-monounsaturated fatty acids. The maximum direct and total incorporation values attained are also greater than for the *cis*-monounsaturated and the straight-chain saturated fatty acid series, being similar to those noted with the iso- and anteiosbranched fatty acid series. It is of interest to note that the *trans*-monounsaturates closely resemble the anteisobranched fatty acids in almost all aspects of their behavior.

The *cis*-polyunsaturated fatty acids tested are relatively poorly incorporated compared to the corresponding 18-carbon fatty acids in all other fatty acid classes tested, with the exception of the C_{18} saturated fatty acid. The introduction of increasing numbers of *cis*-double bonds into an 18-carbon saturated fatty acid results in a progressive and marked decrease in the extent of direct incorporation. The extent of total incorporation also decreases as the number of double bonds present increases, but much less markedly because the extent of chain elongation to their 20-carbon homologues tends to increase with increasing unsaturation, except in the case of arachidonic acid, which does not undergo chain elongation.

The two cyclopropane ring-containing fatty acids tested are incorporated moderately well into the membrane polar lipids. Their extent of direct and total incorporation is greater than that of the straight-chain saturated and polyunsaturated fatty acids of similar chain length but less than that observed for the monounsaturated and branched-chain fatty acids. As noted with the monounsaturated fatty acids, the *trans*-cyclopropane fatty acid is again incorporated to a greater extent than is the corresponding *cis*-isomer. Neither of the cyclopropane fatty acids tested undergoes chain elongation before biosynthetic utilization.

Incorporation of endogenous and exogenous fatty acids into individual glyco- and phospholipids. The five major polar lipids detected in the membranes of *A. laidlawii* B cells were found to be the neutral glycolipids monoglucosyl diglyceride (MGDG) and diglucosyl diglyceride (DGDG), the glycerophosphatides phosphatidylglycerol (PG) and *O*-amino acyl esters of phosphatidylglycerol (*O*-PG), and the acidic glycolipid glycerophosphoryldiglucosyl diglyceride (GPDGDG). When cells are grown in the absence of any exogenous fatty acid, the membrane lipid even-numbered, straight-chain saturated fatty acids, which are derived mainly from de novo biosynthesis (12, 25, 33, 34), are distributed among the various lipid classes in a rather distinctive manner (Fig. 4). The fatty acid compositions of the neutral glycolipids, MGDG and DGDG, are very similar and somewhat different from those of the two phospholipids, PG and *O*-PG, which are also very similar. The neutral glycolipids have increased levels of the C_{12} and C_{14} and reduced levels of the C_{16} and C_{18} saturated fatty acids as compared to the phosphatides. The neutral glycolipids are thus characterized by their proponderance of shorter-chain fatty acids. However, the phosphorylated glycolipid, GPDGDG, has a rather distinctive fatty acid composition quite different from those of both the neutral glycolipids and phospholipids. GPDGDG has a much higher content of C_{12}, a moderate quantity of C_{14}, and much less C_{16} and C_{18} saturates than any of the other fractions. The average chain length of the endogenous fatty acids of GPDGDG is thus much reduced in comparison with the other polar lipids. To determine whether or not these differences in fatty acid composition would persist when the bulk of the membrane lipid fatty acids was derived from exogenous sources, the fatty acid composition of each lipid class from cells grown in a variety of exogenous fatty acid was studied. In some cases, represented here in Fig. 5 by an experiment with heptadecanoic acid as the exogenous acid, the distinctive differences between the neutral glycolipids and phospholipids are retained, and GPDGDG

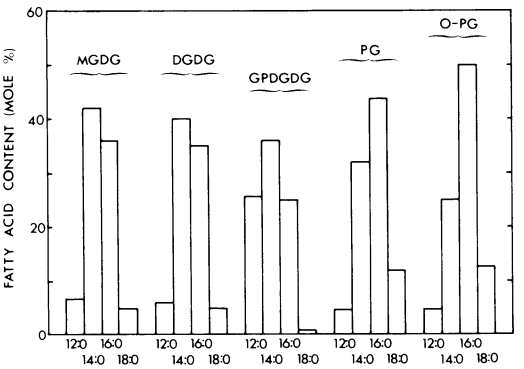

FIG. 4. *Distribution of the major endogenous fatty acids within the five major membrane polar lipids when A. laidlawii B is grown in the absence of any exogenous fatty acids. The fatty acid composition, expressed in mole percent, of each lipid class was determined by GLC; each value presented is the average of at least three independent experiments.*

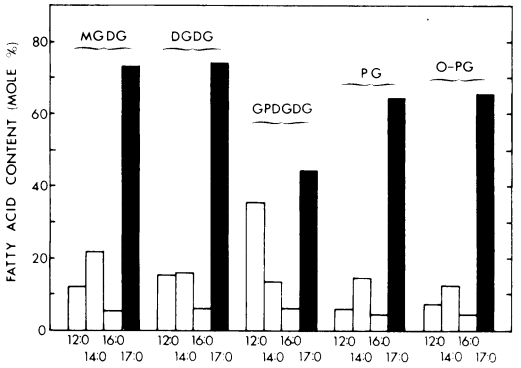

FIG. 5. *Distribution of the exogenous and the major endogenous fatty acids within the five major membrane polar lipids when A. laidlawii B cells are grown in the presence of 0.12 mM heptadecanoic acid. The fatty acid composition, expressed in mole percent, of each lipid class was determined by GLC; each value presented is the average of at least three independent experiments.*

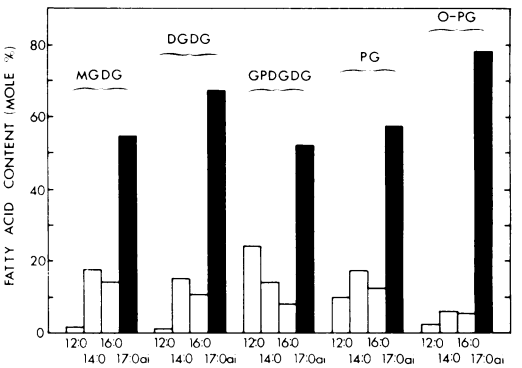

FIG. 6. *Distribution of the exogenous and the major endogenous fatty acids within the five major membrane polar lipids when A. laidlawii B cells are grown in the presence of 0.12 mM anteisoheptadecanoic acid. The fatty acid composition, expressed in mole percent, of each lipid class was determined by GLC; each value presented is the average of at least three independent experiments.*

again shows the peculiar pattern noted previously. In other cases, represented here in Fig. 6 by an experiment with anteisoheptadecanoic acid as the exogenous acid, those distinctive differences between the neutral glycolipids and phospholipids are no longer observed. GPDGDG, however, still exhibits its peculiar pattern, having markedly elevated levels of the C_{12} saturated fatty acid and a somewhat reduced incorporation of the exogenous fatty acid. We could discern no regular relationship between the chemical or physical properties of the exogenous fatty acids tested and their variable distributions between the neutral glycolipid and phospholipid fractions.

DISCUSSION

In the straight-chain saturated fatty acid series, optimum direct incorporation is observed with the 15-carbon acid. When grown in the absence of exogenous fatty acids, this organism produces primarily C_{14} and C_{16} straight-chain saturates (in roughly equal amounts) via de novo fatty acid biosynthesis (12, 25, 33, 34). Since *A. laidlawii* grows well at 35°C without fatty acid supplementation, these saturated fatty acids (average chain length near 15.0) presumably provide an acceptable degree of membrane lipid fluidity. This organism incorporates most effectively those exogenous saturated fatty acids (C_{14} through C_{16}) that most closely resemble the endogenous fatty acids normally produced. For the isobranched, anteisobranched, and *cis*-monounsaturated series, the chain length for optimum direct incorporation is 16, 17, and 18 to 19 carbon atoms, respectively. This is consistent with the predicted effect of methyl branching and *cis*-unsaturation in reducing the "apparent chain length," since the introduction of a methyl isobranch, a methyl anteisobranch, or a *cis*-double bond into a straight-chain saturated fatty acid results in a progressively greater disruption of close molecular packing and decrease in intermolecular attractive interaction (18, 26, 45). This progressive reduction in apolar cohesive force is manifested as a progressive decrease in the gel to liquid-crystalline membrane lipid phase transition temperature and as a progressive increase in membrane lipid fluidity at the optimal growth temperature (18, 26). Although only a limited number of *trans*-monounsaturated fatty acids could be tested, the chain length for optimum incorporation (apparently between 16 and 18 carbon atoms) seems again to be correctly accounted for by the effect of this functional group on the relative apolar attractive forces. The *trans*-double bond would produce an upward shift in the optimum chain length roughly comparable to that produced by the introduction of an anteisomethyl branch. The effect of the introduction of these various functional groups on the direct incorporation of exogenous fatty acids having chain lengths shorter or longer than the optimum can also be explained by the effect of these groups on the cohesive force (roughly the melting temperature). Thus, for any constant but suboptimal chain length (14 carbon atoms or less), the introduction of isobranching, anteisobranching, and *trans*- or *cis*-unsaturation

usually results in a reduced direct incorporation, as would be predicted since the apparent chain, which is already suboptimal, would be reduced still further. Similarly, the introduction of these functional groups into fatty acids having supraoptimal chain lengths (18 carbons or longer) always enhances direct incorporation, again as would be predicted since their introduction would shift the apparent chain length downward toward that needed for maximum incorporation. However, the introduction of two or more *cis*-double bonds apparently reduces the apparent chain length of 18- and 20-carbon fatty acids by too great an extent, since the uptake of polyunsaturates markedly declines with increasing unsaturation. Evidence is presented in the accompanying paper (33) that the increased total incorporation noted with the shortest-chain-length saturated, iso-branched, and anteisobranched fatty acids tested is the result of the utilization of these acids as primers by the de novo fatty acid biosynthetic system present in this organism.

It is interesting to note that when the chain length is optimized, the iso- and anteiso-branched and the *trans*-monounsaturated fatty acid classes are incorporated more extensively than are members of the other fatty acid classes tested. Similar observations have been reported in *Escherichia coli* fatty acid auxotrophs, where the extent of incorporation of exogenous fatty acids is greatest with the *trans*-monounsaturated and progressively decreases as one proceeds through the iso- and anteisobranched, *cis*-monounsaturated, and *cis*-cyclopropane fatty acids to the *cis*-polyunsaturates (8–10, 35, 38–40). It is tempting to speculate that this behavior is due to the fact that certain members of the *trans*-monounsaturated and branched-chain fatty acid series can provide a moderate and optimal level of membrane lipid fluidity, being intermediate in this regard between the high-melting saturated and lower-melting *cis*-monounsaturated, cyclopropane, and polyunsaturated fatty acids. In conjunction with this idea, it might be noted that only *trans*-monounsaturates and iso- and anteiso-branched fatty acids containing 16 to 18 carbon atoms are capable of supporting cell growth of *Mycoplasma* strain Y when this organism, which has an absolute requirement for exogenous fatty acids, is supplemented with any of an extensive series of single saturated, branched-chain, or unsaturated fatty acids (28–30).

The chemical nature of the exogenous fatty acid does not seem to be of major importance in determining its suitability for biosynthetic utilization, since no requirement for the presence of any particular functional group or electronic configuration could be discerned. Also, the marked dependence of incorporation on chain length within any chemical class of exogenous fatty acids supports this view. Recent work with unsaturated fatty acid auxotrophs of *E. coli*, which like *A. laidlawii* B can biosynthesize only saturated fatty acids, has confirmed the relative unimportance of the chemical nature and electronic configuration of the membrane lipid fatty acids (8–10, 35, 38–40).

The incorporation of exogenous fatty acids into membrane lipids is a result of the operation of a complex series of processes, including the permeation or transport of exogenous fatty acids across the cell membrane, the activation of the free acids to coenzyme A or acyl carrier protein derivatives, possibly chain elongation, which itself includes several steps, and the incorporation of the activated fatty acid to the membrane polar lipids. From these in vivo experiments alone, it is difficult to determine which step(s) is responsible for the specificity pattern observed. Studies are presently being conducted with both intact cells and cell-free systems in an effort to characterize the specificity pattern of each component process in this overall scheme. However, it seems clear that the enzyme systems of *A. laidlawii* B that are responsible for the incorporation of exogenous fatty acids function in vivo to maintain the fluidity and physical state of the membrane lipids within a certain broad but limited range (16, 34).

Incorporation of endogenous and exogenous fatty acids into individual glyco- and phospholipids. Unlike most other microorganisms, the cell membrane of *A. laidlawii* B does not contain any nitrogen-containing phospholipids. The three glycolipids (MGDG, DGDG, and GPDGDG) comprise about 55 to 60% of the total lipids (36, 37, 42, 44). The bulk of the remaining membrane lipid, about 35%, is contributed by two phospholipids, PG and *O*-PG, with the former predominating. Thus, the neutral and phosphorylated glycolipids are the predominant class of polar lipids in the membrane of this organism.

A. laidlawii B can synthesize all of its major membrane phospho- and glycolipids, probably via the metabolic pathways outlined in Fig. 7 (1, 3–5, 14, 15, 22–24, 43; J. R. Carter, Fed. Proc. 27:1350, 1968). As in bacteria (see 15 for review), phosphatidic acid serves as a common precursor for all species of membrane lipid. Since the fatty acyl groups in the membrane lipids of *A. laidlawii* B are known to be metabolically stable (13, 20), any fatty acid compositional differences among lipid classes in this

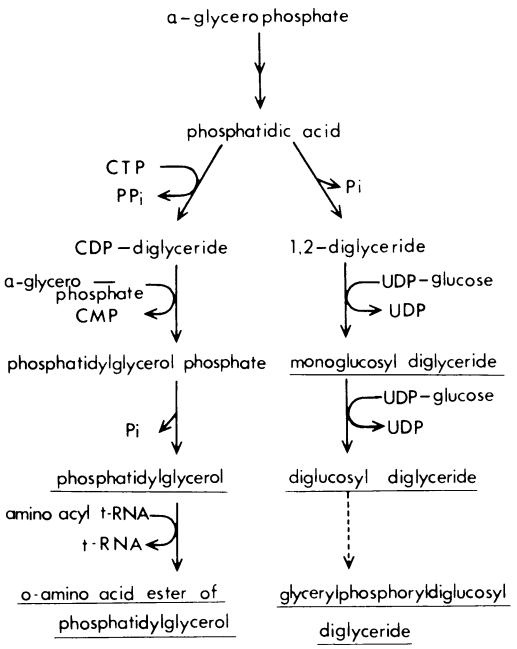

FIG. 7. *Biochemical pathway for the biosynthesis of the five major membrane polar lipids of A. laidlawii B. Abbreviations: CTP, cytidine 5'-triphosphate; Pi, inorganic orthophosphate; PPi, inorganic pyrophosphate; CDP, cytidine 5'-diphosphate; CMP, cytidine 5'-monophosphate; UDP, uridine 5'-diphosphate; tRNA, transfer ribonucleic acid.*

organism have to be generated during lipid biosynthesis, not by deacylation-reacylation reactions (46). When grown in the absence of exogenous fatty acids, the fatty acid compositions of the two neutral glycolipids are very similar, as are the compositions of the two phospholipids. In addition, each class of lipid, the neutral glycolipids, phospholipids, and phosphorylated glycolipids, exhibits a distinct and different fatty acid spectrum. This would indicate that specific phosphatidic acid species are selectively used for cytidine 5'-diphosphate-diglyceride and 1,2-diglyceride synthesis from a common phosphatidic acid pool, or that there are three different phosphatidic acid pools. The latter possibility is perhaps less likely, since all the enzymes involved in complex lipid biosynthesis in this organism are membrane bound, and the existence of cellular compartments in the absence of intracellular membranes is unlikely. However, these differences in fatty acid composition between neutral glycolipids and phospholipids disappear in the presence of some exogenous fatty acids. Since this organism does not always maintain the fatty acid compositional differences between its neutral glycolipids and phospholipids, the difference in composition between these lipids sometimes noted may not be biologically significant. At any rate, even the differences in fatty acid composition between neutral glycolipids and phospholipids observed under certain conditions are not as striking in *A. laidlawii* B as they are in plant and some bacterial systems (45). However, it is interesting to note that GPDGDG maintains in all cases a somewhat unique fatty acid composition different from DGDG, from which it is presumably synthesized (1, 24). This peculiar fatty acid composition of GPDGDG has also been reported by DeKruyff et al. (7). DGDG (or a mixture of MGDG and DGDG), PG, and GPDGDG isolated from *A. laidlawii* B cells grown without exogenous fatty acids or with exogenous elaidic acid all exhibit almost identical gel to liquid-crystalline phase transition temperatures (6, 7). Since under these conditions the fatty acid compositions of each of these fractions are significantly different, it follows that if all fatty acids were randomly distributed these polar lipids would undergo the phase transition at different temperatures. The characteristic differences in the fatty acid compositions of the neutral glycolipids, phosphorylated glycolipids, and the phosphatides may thus function under many conditions to produce a more physicochemically homogeneous mixture of membrane lipids than would result from a random incorporation of endogenous or exogenous fatty acids into each lipid class.

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