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CONTROL OF MEMBRANE POLAR LIPID COMPOSITION IN *ACHOLEPLASMA LAIDLAWII* A BY THE EXTENT OF SATURATED FATTY ACID SYNTHESIS

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Summary

The low level of endogenous fatty acid synthesis in *Acholeplasma laidlawii* A strain EF22 was found to be caused by a deficiency of pantetheine in the lipid-depleted growth medium. By supplementing the oleic acid-containing medium with increasing concentrations of pantetheine, saturated fatty acid synthesis was stimulated (having an apparent K_m of 5 μM for pantetheine) and the incorporation of endogenously synthesized fatty acids in membrane lipids increased markedly. Furthermore, carotenoid biosynthesis was stimulated. Exogenous palmitic acid was found to inhibit partially the endogenous fatty acid synthesis. A gradual stimulation of fatty acid synthesis was accompanied by a linear increase in the molar proportion between the two dominating membrane glucolipids, monoglucosyldiacylglycerol and diglucosyldiacylglycerol. The total amount of charged membrane lipids decreased upon increasing the degree of fatty acid saturation. These regulations are discussed in terms of membrane stability, and influence of membrane molecular ordering and surface charge density on lipid polar head group synthesis.

Introduction

The mycoplasmas have played an important role in membrane molecular biology as tools for elucidating membrane structure and function [1,2]. Much is known about the importance of the physical properties of the fatty acids for the permeability, viscosity and phase transitions of the lipid bilayer [1]. However, very little is known about the function of the different polar head groups of the membrane lipids and the factors that regulate their synthesis.

In a previous investigation we found that the relative amounts of gluco- and phospholipids in the membrane of *Acholeplasma laidlawii* A strain EF 22 varied as a consequence of different fatty acid supplementation to the lipid-depleted but otherwise complex growth medium [3]. In this strain glucolipids comprise more than 50% (mol/mol) of the total membrane polar lipids [3]. The molar ratio between the two major glucolipids, monoglucosyldiacylglycerol and diglucosyldiacylglycerol, was low when the membrane lipids contained only an unsaturated fatty acid, whereas the presence of a saturated fatty acid increased this ratio. This was true for several fatty acids, fatty acid mixtures and cholesterol and it seemed that the physical properties of the fatty acids in the membrane were important for the balance between the glucolipids. This balance could thus possibly depend on the molecular ordering in the membrane. Further evidence in favour of this theory was obtained in temperature-shift experiments [4]. A high degree of molecular ordering resulted in a high monoglucosyldiacylglycerol/diglucosyldiacylglycerol ratio and a lower ordering yielded a lower ratio. The physical properties of monoglucosyldiacylglycerol and diglucosyldiacylglycerol are quite different [5]. Moreover, there was a variation in the degree of lipid fatty acid unsaturation with temperature, indicating that more than one regulatory mechanism was present [4].

When the growth medium is supplied with appropriate unsaturated fatty acids to sustain growth, *A. laidlawii* A strain EF22 has an unusually low level of endogenous saturated fatty acid synthesis compared to other *A. laidlawii* strains [3]. The reason for this has been assumed to be a block or deficiency in saturated fatty acid synthesis. If this syntethic activity could be enhanced, the increased saturated fatty acid content of the lipids, giving a higher molecular ordering, might be yet another means to control the glucolipid amounts in the cell membrane and to more closely examine the relationship between the saturated fatty acid content and the glucolipid ratio. This requires that the endogenously produced fatty acids are utilized in the same way in lipid synthesis as the exogenously incorporated ones. The resulting alteration in molecular ordering could be accomplished without invoking any form of exogenous influence on the organism, as was the case in the previous investigations.

In the present investigation we have therefore made efforts to stimulate the fatty acid biosynthetic machinery in *A. laidlawii.* The effects of pantetheine, a precursor for coenzyme A and acyl carrier protein, on lipid synthesis in *A. laidlawii* A strain EF22 are presented. The results show that the monoglucosyldiacylglycerol/diglucosyldiacylglycerol ratio can be altered as a result of the molecular ordering changes in the presence or absence of endogenous fatty acid synthesis. Furthermore, the amount of charged lipids are dependent upon the degree of fatty acid saturation.

Materials and Methods

Organisms and growth conditions. A. laidlawii A, strain EF22 was used in all experiments. Furthermore, *A. laidlawii* strain B(ju), obtained from Dr. K.-E. Johansson, Uppsala, and B strain JA1 from Dr. A. Liss, N.I.H., Bethesda, MD, were used in control experiments. The organisms were grown in a tryptose/bovine serum albumin medium [4] containing lipid-depleted tryptose [6] and lipid-depleted bovine serum albumin (Cohn V) [6]. However, thallium acetate was not included in the medium. The original procedure for lipid depletion of tryptose was modified by performing the third lipid extraction with chloroform/methanol (2 : 1, v/v) overnight instead of for merely 2 h. The medium was supplemented with fatty acids in ethanolic solutions; 50 μM oleic acid, alternatively 75 μM oleic plus 75 μM palmitic acid (Sigma Chemical Co). Stimulation of the endogenous fatty acid synthesis was achieved by adding pantetheine in concentrations from 0 to 40 μM. In most experiments 12 μM was used. The membrane lipids were labelled by adding 10 μCi/l of [^{14}C]oleic acid (59.7 Ci/mol) and in some experiments 10 μCi/l of [^{14}C]palmitic acid (57.9 Ci/mol), (The Radiochemical Centre, Amersham). To monitor the extent of fatty acid synthesis, 0.20 mCi/l of [^{3}H]acetate (100 Ci/mol, New England Nuclear Co.) was included.

The organisms were subcultured at 37°C in medium with each type of supplementation at least four times for adaptation. For the experiments, a 2% inoculum was grown until late logarithmic phase, i.e. 18 h, and the cells were then harvested. In shift experiments, a 12 h culture was divided in two parts, one of which was supplemented with 12 μM of pantetheine while the other remained unsupplemented. Both cultures were incubated further and growth was estimated by absorbance measurements at 540 nm. Samples were withdrawn for membrane lipid and protein analysis at intervals. Cells were harvested by centrifugation at 32 000 $\times g$ for 10 min at 5°C in a Beckman J21 centrifuge and washed once in β-buffer [4]. Membranes were prepared, washed and freeze-dried according to [4].

Lipid analysis. Membranes were extracted and the lipid extracts were purified exactly as described before [4]. Parts of the extracts were taken to dryness and methylated [3]. Fatty acid composition was determined by gas-liquid chromatography [3]. Furthermore, the fatty acid composition of uninoculated growth medium was determined.

The individual lipid species were separated by thin-layer chromatography on silica gel H (Merck) plates (buffered with 1% (w/v) $Na_2B_4O_7 \cdot 10H_2O$) in chloroform/methanol/water (65 : 25 : 4, v/v) for polar lipids, or on unbuffered plates in petroleum ether (b.p. 40—60°C)/diethyl ether/acetic acid (80 : 20 : 1, v/v) for neutral lipids. Lipids were visualized by iodine vapour. The lipid-containing zones were scraped off the plates and the lipids were eluted into scintillation vials as described [4]. After evaporation of the solvent, scintillation cocktail was added (Omnifluor, New England Nuclear Co.) and radioactivity was determined by liquid scintillation counting [4].

The amounts of unsaturated (oleic) acid relative to saturated fatty acids were determined by gas chromatography. By combination with the data from liquid scintillation counting, an estimation of the molar amounts of exogenously incorporated and endogenous synthesized fatty acids in the total lipid extracts and in the individual lipid species could be calculated. The ratio of unsaturated to saturated fatty acids in the lipids are almost the same with as well as without pantetheine (Table I). In the presence of exogenous oleic plus palmitic acids and pantetheine, the total amounts of endogenously synthesized fatty acids were estimated by assuming that the molar ratio of oleic/palmitic acids (exogenously incorporated) is the same as in cells grown without pantetheine.

Analytical methods. Protein was determined by the method of Lowry et al. [7] using bovine serum albumin (Cohn V) as a standard.

Carotenoids were tentatively identified by spectrophotometry wave-length scanning in hexane solutions.

The membrane protein patterns of the different cultures were analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis [8].

Results

Endogenous saturated fatty acid synthesis in different A. laidlawii strains

A. laidlawii strain A has an absolute requirement for an unsaturated fatty acid, which it cannot synthesize itself [9], whereas the B strain can manage without unsaturated fatty acids [10]. Saturated fatty acids can, however, be synthesized by both strains. The requirement for unsaturated fatty acids was verified for our strain EF22 as before [3]. Addition of pantetheine to media containing saturated fatty acids only, did not make the growth medium able to sustain growth of *A. laidlawii* A (EF 22). Therefore, oleic acid was always included in the following experiments.

In order to assess the efficiency of the lipid extraction procedure for the growth medium components, residual fatty acids from uninoculated lipid-extracted medium containing 75 μM oleic acid were analyzed by gas-liquid chromatography. Less than 4% (mol/mol) of the membrane fatty acids were of medium origin. When cells were grown in this medium, total lipid extracts from strain A (EF 22) contained 3.6% fatty acids other than oleic acid, strain B(ju) 4.0% and strain B(JA1) 17% other fatty acids. The JA1 polar membrane lipid composition differs from that of the other strains (Steinick, L.E., personal communication).

Fatty acid synthesis in *A. laidlawii* proceeds via the malonyl-CoA pathway, employing acetate as a precursor [11]. [^{3}H]Acetate was incorporated into fatty acids under these conditions, indicating that a low level of endogenous synthesis was present. We did not attempt to discriminate between the minimal amounts of fatty acids originating from the medium and endogenously produced fatty acids in the further experiments with *A. laidlawii* A (EF22). Therefore endogenous synthesis in the following results is actually a slight overestimation of this capacity, since residual medium fatty acids are included.

All strains exhibited a lower degree of endogenous saturated fatty acid synthesis than reported earlier for *A. laidlawii* in lipid-depleted oleic acid-containing media [12,13]. The low level of endogenous synthesis is thus not an exclusive property of *A. laidlawii* A (EF22). The main reason for the diminished capacity of fatty acid synthesis seems to be a consequence of the procedure for medium preparation.

Regulation of saturated fatty acid synthesis by pantetheine and palmitic acid in A. laidlawii A (EF22)

The results obtained above indicate a deficiency in the growth medium of some component necessary for fatty acid synthesis. We have shown earlier that this was not due to a lack of acetate [3]. Pantetheine is known to be a biosynthetic precursor for the prosthetic groups of coenzyme A and acyl carrier protein, both of which are needed in fatty acid synthesis. Pantetheine stimulated

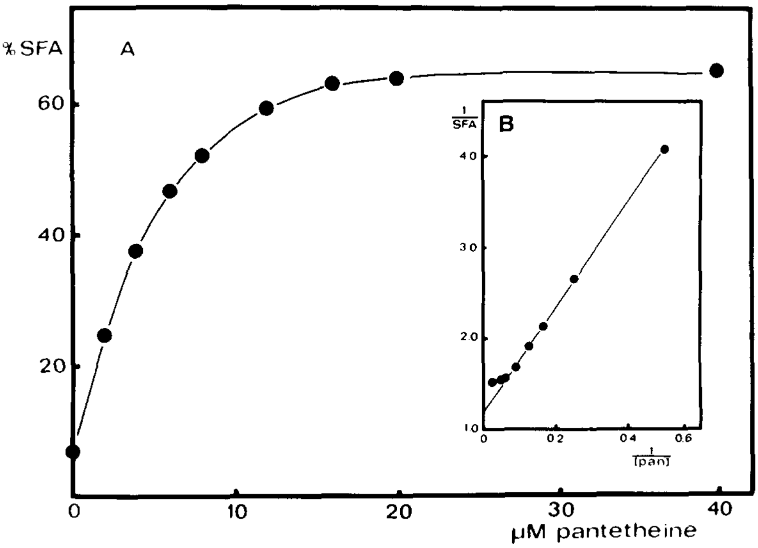


Fig. 1. Effect of pantetheine concentration on the extent of endogenous saturated fatty acid synthesis in *A. laidlawii* A (EF22). Cultures were grown at 37°C for 18 h in a lipid-depleted bovine serum albumin/ tryptose medium supplemented with 50 μM of oleic acid. Fatty acids were analyzed by gas-liquid chromatography. (A) Saturated fatty acid (SFA) content (mol/100 mol) of membrane lipids at different concentrations of pantetheine. (B) (Inset) Lineweaver-Burk plot of the data in (A).

acyl carrier protein activity in *A. laidlawii* A (oral strain) [14] when grown in a partly defined medium [15].

Fig. 1 shows the effect of increased pantetheine concentrations on the content of saturated fatty acids in the membrane lipids. Obviously lack of pantetheine limits fatty acid synthesis in the unsupplemented medium and an increase from approx. 5% to 65% (mol/mol) saturated fatty acids was observed upon pantetheine addition. Furthermore, cell yield increased by 75% as measured by absorbance at 540 nm. Usually 150 μM of exogenous fatty acids are added to give optimal growth [3]. Consequently 50 μM oleic acid will limit growth and a stimulation of fatty acid synthesis will increase growth. Fig. 1A indicates that the synthesis of saturated fatty acids obeyed saturation kinetics and a Lineweaver-Burk plot (Fig. 1B) confirmed this, giving an apparent K_m of 5 μM. Different batches of substrate gave K_m values ranging from 1 to 5 μM, probably due to differences in the original pantetheine concentration.

If end-product regulation exists, the presence of exogenous saturated fatty acids would affect the endogenous synthesis. The effects of pantetheine supplementation in medium with either 75 μM oleic acid or 75 μM oleic plus 75 μM palmitic acid was therefore investigated (Table I). As before, growth increased markedly in the oleic acid cultures containing pantetheine. A small increase in cellular lipids was also evident in the palmitic/oleic acid plus pantetheine medium. In the oleic acid medium a low level of endogenous saturated fatty acid synthesis was apparent in the absence of pantetheine whereas pantetheine supplementation increased this synthesis dramatically. In the oleic plus palmitic acid medium there was a likewise low biosynthetic activity, but the effect of pantetheine addition in this case was a significantly smaller increase in syn-

TABLE I

INCORPORATION OF ENDOGENOUSLY SYNTHESIZED AND EXOGENOUSLY SUPPLIED FATTY ACIDS INTO THE MEMBRANE LIPIDS OF *A. LAIDLAWII*. INFLUENCE OF PANTETHEINE AND PALMITIC ACID

Oleic ($18:1_c$) and palmitic (16:0) acids were added each at a concentration of 75 μM, pantetheine (pan) at 12 μM. Cells were grown for 18 h. Exogenously added fatty acids were labelled with ^{14}C and endogenous synthesis was monitored by the incorporation of [^3H]acetate. Analysis was made by liquid scintillation counting and gas-liquid chromatography. SFA, saturated fatty acids.

Supplementation to the growth medium	nmol of SFA synthesized	nmol of exogenous fatty acids incorporated	% endogenous SFA in lipids	Total % SFA in lipids
$18:1_c$	27.7	466.5	5.6	5.6
$18:1_c$ + pan	984.4	670.8	59.5	59.5
16:0 + $18:1_c$	99.7	1326.2	7.0	56.6
16:0 + $18:1_c$ + pan	357.8	1440.2	22.6	62.6

thesis. These data suggest a possibility to control the activity of the fatty acid biosynthetic machinery at will, by the use of pantetheine and palmitic acid.

Effects of an increased fatty acid synthesis on the synthesis of membrane lipid species

Acetate is a normal catabolite in *A. laidlawii* [16]. Since the size of the endogenous acetate pool is difficult to estimate, it was only possible to use the incorporation of [^3H]acetate as a qualitative measurement of endogenous saturated fatty acid synthesis. The fatty acids were incorporated into all polar lipids as well as into the neutral lipid fraction. In order to estimate membrane polar lipid composition we used the incorporation data for exogenously added fatty acids, since the exact amounts of both labelled and unlabelled acids were known.

Fig. 2. shows how the membrane molar ratio monoglucosyldiacylglycerol/diglucosyldiacylglycerol was altered in cultures grown for 18 h in media with

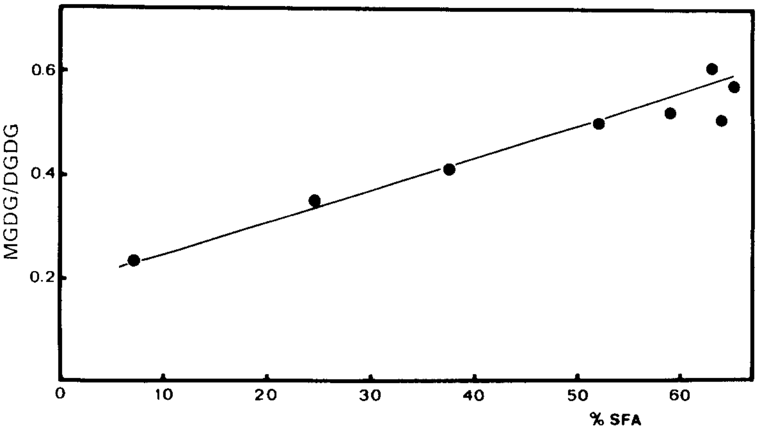


Fig. 2. Molar ratio between monoglucosyldiacylglycerol (MGDG) and diglucosyldiacylglycerol (DGDG) for membranes with different amounts of endogenously produced saturated fatty acids. The membranes were obtained by growing cells for 18 h in 50 μM oleic acid medium supplemented with different concentrations of pantetheine. Lipid amounts were estimated by liquid scintillation counting.

different pantetheine concentrations. These thus have different degrees of fatty acid saturation (cf. Fig. 1). Under the growth conditions employed, there was a linear relationship between the percentage of saturated fatty acids in the membrane lipids and the glucolipid ratio,

The ratios in Fig. 2 were compared with the ratios obtained in the presence of exogenous palmitic acid (Table II). An increase in the content of saturated fatty acids (Table I) yielded a higher monoglucosyldiacylglycerol/diglucosyldiacylglycerol ratio (Table II) regardless of the origin of the fatty acids. The values obtained are in good agreement with the results in Fig. 2. Thus there is no qualitative difference in the effect of fatty acids from different sources.

In addition to the dominating, electrically neutral (actually weakly anionic [5,17]) glucolipids, the membrane of *A. laidlawii* contains charged (strongly anionic) lipids, i.e. phosphatidylglycerol and glycerophosphoryl derivatives of mono- and diglucosyldiacylglycerol [3]. Table II reveals that when increasing the degree of fatty acid saturation in the lipids, there was a decrease in the total amounts of charged lipids. The same relationship was found after a successive increase in the saturated fatty acid content by supplementation of pantetheine to oleic acid medium (data not shown) (cf. Fig. 1).

When grown under normal conditions, *A. laidlawii* contains trace amounts of a glucolipid X (containing glycerol, glucose and fatty acids in the proportion 1 : 1 : 4) [2,3]. When grown in 50 μM oleic acid medium with 12 μM pantetheine, lipid X increased to approximately 10% (mol/mol) of the polar lipids. Typically this lipid always contained a very high degree of saturated fatty acids (approx. 90% mol/mol).

In the presence of pantetheine in the growth medium the cell membrane of *A. laidlawii* was brilliantly yellow in contrast to the greyish-white colour in its absence. The coloured compound was a neutral lipid and was identified as a carotenoid, probably neurosporene, on basis of its absorption spectrum in hexane (absorption maxima at 405, 432, 467 nm) (cf. Ref. 18). Examination of the other neutral lipids revealed an increase in the proportion of free fatty acids of endogenous origin. The total proportion of neutral lipids in the membrane did not increase substantially.

TABLE II

EFFECT OF PANTETHEINE AND PALMITIC ACID ON THE SYNTHESIS OF GLUCOLIPIDS AND CHARGED LIPIDS

The values refer to the same experiment as in Table I. See Table I for abbreviations. Molar ratio MGDG/DGDG is the molar ratio between monoglucosyldiacylglycerol (MGDG) and diglucosyldiacylglycerol (DGDG). The lipids used for the percent charged lipids of total data are: glycerophosphoryl monoglycosyldiacylglycerol, glycerophosphoryl diglucosyldiacylglycerol and phosphatidylglycerol.

Supplementation to the growth medium	Molar ratio MGDG/DGDG	% charged lipids of total (mol/mol)
$18{:}1_c$	0.26	28.5
$18{:}1_c$ + pan	0.53	24.5
$16{:}0 + 18{:}1_c$	0.62	23.1
$16{:}0 + 18{:}1_c$ + pan	0.68	21.9

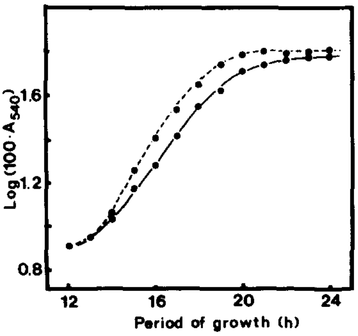
The lipids used for the percent charged lipids of total data are: glycerophosphoryl monoglycosyldiacylglycerol and phosphatidylglycerol.

Induction of endogenous fatty acid synthesis by pantetheine during growth

The previous experiments all indicated that pantetheine supplementation to the lipid-depleted growth medium stimulated the synthesis of saturated fatty acids. The resultant increase in membrane molecular ordering [19] was accompanied by an increase in the monoglucosyldiacylglycerol/diglucosyldiacylglycerol ratio. The effects of temperature on this ratio has been investigated earlier in temperature-shift experiments [4]. Analogous experiments were now performed with pantetheine. A 50 μM oleic acid culture was grown at 37°C for 12 h. At this time the culture was divided in two parts. One part was supplemented with 12 μM pantetheine and the other remained unsupplemented. After supplementation there was an immediate increase in the rate of growth of the pantetheine culture (Fig. 3) and the cell membranes rapidly turned yellow. The saturated fatty acid content of the lipids also increased from 6 to 61% (mol/mol) in 12 h (data not shown), whereas there was a decrease in fatty acid saturation in the unsupplemented culture. Fig. 4 shows the changes in the monoglucosyldiacylglycerol/diglucosyldiacylglycerol ratio during growth. Obviously the same response in the ratio can be obtained by stimulation of endogenous saturated fatty acid synthesis in *A. laidlawii* as was provoked by a rapid temperature decrease.

Membrane protein composition

Sodium dodecyl sulphate polyacrylamide electrophoresis revealed small differences in staining intensity for a few bands in cultures grown with different fatty acid supplementations as shown earlier [8]. However, addition of pantetheine during growth did not result in detectable changes in membrane protein patterns.



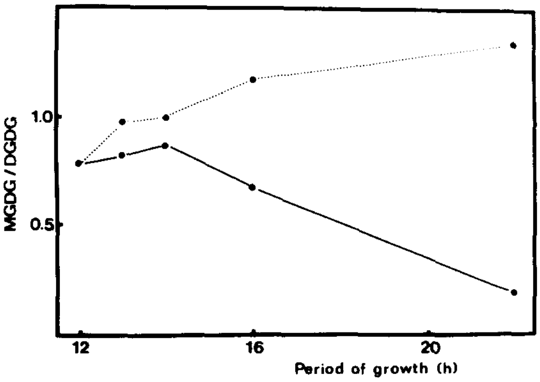


Fig. 3. Growth-promoting effect of pantetheine. A 50 μM oleic acid culture was divided in two parts after 12 h of growth at 37°C. One half was supplemented with 12 μM of pantetheine (hatched line) and the other remained unsupplemented (———). Growth was estimated by absorbance measurements at 540 nm.

Fig. 4. Changes in the molar ratio monoglucosyldiacylglycerol/diglucosyldiacylglycerol after stimulation of endogenous saturated fatty acid synthesis. After 12 h of growth at 37°C in medium containing 50 μM oleic acid, half of the culture was supplemented with 12 μM pantetheine (· · · · · ·) while the other half remained unsaturated (———). Membrane polar lipid composition was analyzed at the times indicated (see Materials and Methods).

Discussion

A. laidlawii is capable of synthesizing saturated fatty acids from acetate in complex media [9]. From the results obtained with different *A. laidlawii* strains in our lipid-depleted medium, it is obvious that pantetheine can be a limiting factor for fatty acid synthesis, not only in partially defined media [14], but also in lipid-depleted complex media. By the use of extraction procedures which deplete the medium of pantetheine it is possible to virtually stop the endogenous saturated fatty acid synthesis. This procedure should make it possible to apply isotope techniques for accurate determination of fatty acid and lipid composition of many *A. laidlawii* strains. The method has been successfully used for *A. laidlawii* A (EF22) [3,4]. Inhibitors of fatty acid synthesis have been used to decrease endogenous synthesis and increase incorporation of exogenous fatty acids into *Acholeplasma* in studies of growth and fatty acid composition [20,21]. The use of a pantetheine-deficient medium may become a valuable complementary method in studies of lipid metabolism, since many inhibitors have toxic effects.

The mechanism of saturated fatty acid synthesis is well established for many organisms, e.g. *Escherichia coli* [22]. The malonyl-CoA pathway is probably at work in *A. laidlawii* too [11]. Acyl carrier protein plays an important role in this pathway, and a precursor, pantetheine, was found to stimulate acyl carrier protein activity of *A. laidlawii* substantially in a partially defined medium [14]. This probably occurred in our growth medium too, and a Lineweaver-Burk plot (Fig. 1b) revealed an apparent K_m of 5 μM for the influence of pantetheine on fatty acid synthesis. For comparison, a pantothenate auxotropic *E. coli* strain was found to require 2 μM pantothenate to maintain exponential growth [23]. After addition of pantetheine during growth of *A. laidlawii* (Figs. 3 and 4) the onset of fatty acid synthesis was rapid, indicating that fatty acid synthetase may be a constitutive enzyme complex. Exogenous palmitate inhibited fatty acid synthesis in *A. laidlawii* A (EF22), confirming previous investigations on other strains [24,25]. An end-product regulation of fatty acid synthesis thus exists, although the efficiency of this mechanism has recently been challenged [25].

The addition of pantheteine resulted in a stimulation of carotenoid biosynthesis and the membranes turned yellow. Pantetheine is a precursor for coenzyme A, which in turn is needed in carotenoid biosynthesis. In media with pantetheine deficiency, the existing CoA is obviously spared for more important activities than carotenoid biosynthesis, which implies that carotenoids are not essential membrane components under all circumstances. Carotenoids are known to increase the molecular ordering in *A. laidlawii* membranes [26] and this has been suggested as a possible mechanism for a partial regulation of membrane fluidity [27].

Stimulation of the endogenous saturated fatty acid synthesis has a profound impact on polar lipid synthesis in *A. laidlawii.* Fig. 2 demonstrates the tightly coupled relationship which exists between the degree of fatty acid saturation and the monoglucosyldiacylglycerol/diglucosyldiacylglycerol ratio under the conditions described. These data give further evidence for the sensitivity of this lipid polar head regulatory mechanism. The regulation is, however, not sensitive to fatty acid saturation per se, since a decrease in temperature during growth [4] gives qualitatively the same increase in the glucolipid ratio as the stimulation of saturated fatty acid synthesis caused by pantetheine (Fig. 4). Moreover, the glucolipid ratio is not coupled to any specific growth phase (Fig. 4 and Ref. 4). These experiments therefore indicate that the regulation mechanism senses the molecular ordering in the membrane. X-ray diffraction and ^2H_2O NMR measurements revealed that monoglucosyldiacylglycerol and diglucosyldiacylglycerol differ considerably in physical properties. Thus, pure monoglucosyldiacylglycerol exists as a non-bilayer phase (reverse hexagonal), whereas diglucosyldiacylglycerol has a lamellar phase structure [5]. Membrane lipid mixtures have lamellar phase appearances, this being the only phase structure compatible with the existence and function of biological membranes. It is possible that the regulation mechanism in *A. laidlawii* might be a similar method to regulate membrane stability of this mixture of components with different phase behaviours, as recently suggested for phosphatidylethanolamine from natural sources [28]. Phosphatidylethanolamine can change between different phase structures depending on temperature and fatty acid composition [28]. In light of its high saturated fatty acid content, the appearance of the apolar monoglucolipid X (which has a lamellar phase structure [5]) might be yet another regulating or scavenging mechanism (cf. [3]). Evidence for the existence of mechanisms sensitive to membrane molecular ordering have been obtained in *Tetrahymena pyriformis* for the fatty acid desaturase, which is regulated by membrane fluidity [29]. Furthermore, the importance of the lipid polar head groups for the regulation of membrane fluidity has been recently demonstrated for murine fibroblasts (LM cells) [30]. LM cells are uncapable of synthesizing phosphatidylcholine from phosphatidylethanolamine. Supplementation of the culture with different choline analogues resulted in incorporation of these precursors and fatty acid compositions intermediate to ethanolamine and choline-supplemented cultures. A reverse mechanism sensitive to molecular ordering and affecting polar lipid head group composition is equally possible in *A. laidlawii.* Furthermore, differences in growth temperature affected the proportions of the individual phospholipids in *Pseudomonas halosaccharolytica* [31], giving further strength to the concept that different polar head groups may have important regulatory functions in membranes.

The degree of fatty acid saturation of *A. laidlawii* lipids also affected the proportion of charged lipids in the membrane (Table II). An increase in the amount of *cis*-unsaturated fatty acids (i.e. oleic acid) into the lipids makes the lipid molecules more bulky in the apolar part, probably leading to a decrease in membrane stability (cf. Ref. 32). Furthermore, unsaturated lipids will increase the lipid lateral packing area [33] and a decrease in the surface charge density will occur [34]. It is possible that the cell compensates for these changes by an increased synthesis of charged lipids, thereby restoring surface charge density and bilayer stability. Experimental evidence for this proposed mechanism in strain EF22 has indeed been observed after fatty acid manipulation [3] as well as temperature shiftdown [4]. In mutants of *Neurospora crassa* defective in lipid synthesis, it was found that albeit great differences in lipid composition in different mutants, the ratio of zwitterionic (weakly anionic) to the anionic lipids was always constant [35], indicating the importance of constant membrane charge. Furthermore, the same balance seems to be of vital importance in *E. coli* [36]. The concept of constant surface charge density in *A. laidlawii* will be further investigated in future experiments. However, our results indicate that membrane polar lipid composition is regulated on at least two levels, i.e. the monoglucosyldiacylglycerol/diglucosyldiacylglycerol ratio and the the balance between neutral and charged lipids.

In conclusion, the opportunity to control fatty acid synthesis *A. laidlawii* by the addition of pantetheine constitutes a powerful tool for investigations on the regulation and role of different lipid polar head groups in these membranes.

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