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Difference in packing properties between iso and anteiso methyl-branched fatty acids as revealed by incorporation into the membrane lipids of *Acholeplasma laidlawii* strain A

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***Acholeplasma laidlawii* strain A was grown on the long-chain, branched, saturated fatty acids 12-methyltetradecanoic acid (anteiso-C_{15}), 14-methylhexadecanoic acid (anteiso-C_{17}), 13-methyltetradecanoic acid (iso-C_{15}) and 15-methylhexadecanoic acid (iso-C_{17}), and on the branched-chain fatty acid precursors 2-methylbutanoic acid (anteiso-C_5) and 3-methylbutanoic acid (iso-C_5). The membrane lipid composition as a function of the fatty acid supplement and the growth temperature was determined: (1) The ratio between the two dominating lipids, monoglucosyldiacylglycerol and diglucosyldiacylglycerol, is decreased when anteiso acyl chains replace iso acyl chains in the lipids, and when the acyl chains are elongated from 15 to 17 carbon atoms; (2) the ratio iso/anteiso acyl chains is reduced when the growth temperature is decreased; (3) the average length of the de novo synthesized branched fatty acids is decreased when the growth temperature is lowered, and when anteiso-C_5 is exchanged for iso-C_5 in the growth medium. The lipid regulation mechanisms are interpreted in terms of lipid molecular geometry and self-assembly of lipid molecules. It is concluded that iso and anteiso fatty acids have distinctively different packing properties in a biological membrane, and they mimic straight-chain saturated and *cis*-monounsaturated fatty acids, respectively, as seen by the way they affect the physicochemical properties of membrane lipids and by the way they are used in lipid regulation mechanisms.**

Introduction

Several genera of Gram-positive and Gram-negative bacteria have membrane lipids which contain mainly iso and anteiso methyl-branched saturated acyl chains [1]. The two kinds of fatty acids have different physicochemical properties: anteiso acids have lower melting points [1] and occupy larger areas in a monolayer at the air-water interface [2], than do iso acids of the same chain length.

The influence of iso and anteiso acyl chains on the packing properties of a membrane lipid has been studied. Phosphatidylethanolamine (PE) was isolated from *Bacillus megaterium* grown at different temperatures, and the phase equilibria in PE-water mixtures were determined [3]. At 60°C and at low water contents, PE with a low ratio of iso/anteiso acyl chains forms a cubic liquid-crystalline phase, while PE with a 10-fold higher value of this ratio forms a lamellar liquid-crystalline phase. The molecular shape of membrane lipids, and hence the ability to form various aggregate

Abbreviations: PE, phosphatidylethanolamine; MGDG, monoglucosyldiacylglycerol; DGDG, diglucosyldiacylglycerol; PG, phosphatidylglycerol; GPMGDG, GPDGDG, glycerophosphoryl derivatives of MGDG and DGDG.

structures, is thus affected by the position of the methyl-branching point along the acyl chain [3,4]. The difference in molecular shape between lipids containing iso and anteiso acyl chains is most probably an important factor in a membrane lipid regulation mechanism operating in *B. megaterium*: the ratio iso/anteiso acyl chains is raised by more than a factor of 10 when the growth temperature is increased from 5 to 70°C [5]. The formation of an unstable lipid bilayer, or even a non-lamellar lipid phase, at higher temperatures is avoided by raising the ratio iso/anteiso acyl chains.

In this work the function and packing properties of iso and anteiso acids in biological membranes are further elucidated through the incorporation of these acids into the membrane lipids of *Acholeplasma laidlawii*. This organism is frequently used for studies of membrane lipid organization and dynamics [6]. It has been shown that *A. laidlawii* alters the polar head group composition of the membrane lipids upon: (1) incorporation of different straight-chain saturated and unsaturated fatty acids; (2) changes is growth temperature; and (3) incorporation of cholesterol and anesthetics into the membrane [7–9]. The regulation of the lipid composition has been interpreted as an effort to maintain optimal packing of the bilayer structure [4,7,10], and the discussions are based on recent developments of the theory of self-assembly of amphiphiles [11,12]. The validity of this interpretation is supported by investigations of the phase structures formed by mixtures of the two dominating lipids in the *A. laidlawii* membrane, monoglucosyldiacylglycerol (MGDG) and diglucosyldiacylglycerol (DGDG), in the absence and presence of cholesterol [13,14]. This new explanation of membrane lipid regulation mechanisms in *A. laidlawii* has up to now been based on experiments in which straight-chain fatty acids were incorporated into the lipids. It is shown here that the self-assembly theory is applicable when the organism incorporates branched-chain fatty acids as well.

A. laidlawii strain B has been shown to incorporate long branched-chain fatty acids [15,16] as well as branched-chain fatty acid precursors [17] into the membrane lipids. To the author's knowledge this is the first report on the growth of *A. laidlawii* strain A on these fatty acids. It has been asserted that strain A, in contrast to strain B, is dependent on a long-chain unsaturated or a cyclopropane fatty acid for growth [18–21]. However, it is demonstrated here that strain A grows equally well when these fatty acids are replaced by certain branched-chain fatty acids.

Materials and Methods

Organism and growth conditions. A. laidlawii strain A (EF22) [22] was used in all experiments. The organism was grown in a medium containing per litre: 20 g lipid-depleted tryptose [23], 4 g lipid-depleted bovine serum albumin (Cohn V) [23], 7 g glucose, 5 g NaCl, 5 g Tris and 100 000 I.U. penicillin G. The medium was supplemented with fatty acids in ethanolic solutions: (1) 120 μM of one of 12-methyltetradecanoic (anteiso-C_{15}), 14-methylhexadecanoic (anteiso-C_{17}), 13-methyltetradecanoic (iso-C_{15}) or 15-methylhexadecanoic (iso-C_{17}) acids; (2) different equimolar mixtures of anteiso-C_{15}, anteiso-C_{17}, iso-C_{15} and iso-C_{17}, the total concentration being 120 μM; (3) 12 μM of pantetheine together with 1.0 mM of either 2-methylbutanoic acid (anteiso-C_5) or 3-methylbutanoic acid (iso-C_5), or together with a mixture of these acids (0.5 mM each).

Cells were cultured at 27 and 37°C, and harvested in the late logarithmic or early stationary phase of growth 16–22 h after inoculation. The organism was subcultured at least five times in medium with each type of supplementation and at the proper temperature before the final cultivation. Cell growth was monitored by measuring the turbidity at 540 nm.

Membrane preparation and lipid extraction. Cells were harvested and membranes were prepared as described earlier [24]. Each membrane preparation was extracted with 14 ml of chloroform/methanol (2 : 1, v/v) for 1 h at room temperature, 0.7 ml of double distilled water was added, and the lipid extracts were purified from non-lipid contaminants by passage through a Sephadex® G-25 Fine column [25]. The solvents were evaporated at 35°C under a stream of nitrogen and the lipids were finally dissolved in a small volume of chloroform/methanol (2 : 1, v/v).

Determination of polar head group and acyl chain composition. In the experiments with the long

branched-chain fatty acids the different lipids were quantified by gas-liquid chromatography (GLC) after separation by thin-layer chromatography (TLC) [22]. Lipid spots on the TLC plates were visualized with rhodamine 6G and scraped into Pasteur pipettes stoppered with glass wool. The lipids were eluted with methanol/chloroform (9 : 1, v/v) into screw-capped test tubes, and the solvents were evaporated at 35°C under a stream of nitrogen. Lipid acyl chains were converted to their methyl esters as described [5]. A known amount of methyl elaidate was added as internal standard to each sample. The GLC analyses were performed with a Varian model 3700 apparatus equipped with a glass column, 2.0 m long, 1.3 mm in diameter, packed with 10% SP-2330 on Supelcoport, 100/120 mesh. The GLC apparatus was equipped with a Hewlett-Packard model 3390A electronic integrator.

When *A. laidlawii* was grown on the branched-chain fatty acid precursors the membrane lipids were labelled by adding 0.40 mCi/l of sodium [^{3}H]acetate to the medium. The lipids were separated by TLC and eluted into scintillation vials as described above. After evaporation of the solvents a scintillation cocktail was added, and the radioactivity was determined by liquid scintillation counting [24]. The acyl chain composition was determined in total lipid extracts by GLC.

Materials. The long branched-chain fatty acids (purity $\geq$ 98%) were obtained from Larodan Fine Chemicals, Malmö, Sweden. Pantetheine (purity 98%) was purchased from Serva Feinbiochemica, Heidelberg, F.R.G.; 2-methylbutanoic acid (purity 98%) from EGA-Chemie, Steinheim, F.R.G., and 3-methylbutanoic acid (purity 99%) from Sigma Chem. Co., St. Louis, MO., U.S.A. Sodium [^{3}H]acetate was from New England Nuclear, Dreieich, F.R.G. The Sephadex® G-25 Fine gel was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden.

Results

Fatty acid growth requirement of A. laidlawii strain A

It has been argued that *A. laidlawii* strain A has an absolute growth requirement for an unsaturated fatty acid with 16 or 18 carbon atoms [18–20,22], or a cyclopropane acid with 17 or 19 carbon atoms [20,21]. However, anteiso-C_{15}, anteiso-C_{17} and iso-C_{15} all supported good growth of strain A; iso-C_{17} was less efficient in supporting growth. The organism was able to grow on the branched-chain fatty acid precursors anteiso-C_5 and iso-C_5 as well, if these acids were added in combination with pantetheine to the medium. Due to the efficiency of the lipid extraction of tryptose and bovine serum albumin, pentetheine must be added to the growth medium in order to make the organism capable of de novo synthesis of long saturated fatty acids [23].

Polar head group composition of membrane lipids

Five polar lipids always occur in the membranes of *A. laidlawii* strains A and B [9,22]: MGDG, DGDG, phosphatidylglycerol (PG), and glycerophosphoryl derivatives of MGDG and DGDG (GPMGDG and GPDGDG). A sixth polar lipid, glucolipid X, is synthesized in increasing amounts by the organisms when the fraction of straight saturated acyl chains in the membrane lipids is increased [22–24,26,27]. Glucolipid X has been suggested to be composed of two diacylglycerol molecules linked to one glucose molecule [22,28].

Growth of *A. laidlawii* strain A on different iso and anteiso fatty acids resulted in marked differences in the ratio between MGDG and DGDG (Table I). These lipids have different packing properties, MGDG forming a reversed hexagonal (H_{II}) phase and DGDG a lamellar phase in excess water [13,14,29]. The ratio MGDG/DGDG has been shown to vary when straight-chain saturated and unsaturated fatty acids are incorporated in different proportions into the *A. laidlawii* membrane lipids [7,9]. Incorporation of iso fatty acids gave higher values of this lipid ratio than incorporation of anteiso fatty acids; the difference was more pronounced with the long-chain fatty acids. Elongation of these fatty acids from 15 to 17 carbon atoms resulted in a reduction of the MGDG/DGDG ratio. Finally, when the growth temperature was lowered from 37 to 27°C a marked increase in this lipid ratio was obtained (data not shown).

The balance between ionic (PG, GPMGDG and GPDGDG) and non-ionic (MGDG and

TABLE I

GLUCOLIPID COMPOSITION IN MEMBRANE LIPID EXTRACTS FROM *ACHOLEPLASMA LAIDLAWII* A GROWN ON LONG-CHAIN BRANCHED FATTY ACIDS AND BRANCHED-CHAIN FATTY ACID PRECURSORS

The long-chain fatty acids were added at a concentration of 120 μM. The fatty acid precursors were added at a concentration of 1.0 mM when supplied alone, and at 0.5 mM each when supplied together. When cells were grown on the fatty acid precursors the growth medium was also supplemented with 12 μM of pantetheine (see text for explanation). Cells were grown at 37°C. i, iso; a, anteiso; C_n, number of carbon atoms. Results are given as molar ratio monoglucosyldiacylglycerol/diglucosyldiacylglycerol ± S.E. of the number of independent experiments given in parentheses.

Fatty acid supplement	MGDG/DGDG
i-C_5	1.35 ± 0.03 (4)
i-C_5/a-C_5	1.15 ± 0.02 (4)
a-C_5	0.95 ± 0.02 (4)
i-C_{15}	2.9 ± 0.2 (3)
i-C_{15}/a-C_{15}	1.62 ± 0.17 (3)
a-C_{15}	0.80 ± 0.03 (4)
i-C_{17}	2.3 ± 0.2 (3)
i-C_{17}/a-C_{17}	0.66 ± 0.03 (3)
a-C_{17}	0.66 ± 0.02 (4)

DGDG) lipids in the *A. laidlawii* membrane is varied when different proportions of straight-chain saturated and unsaturated fatty acids are incorporated into the lipids [7,9]. This balance was affected also when the organism was fed with different branched-chain fatty acids. The ionic lipid fraction was 28 mol% when the organism was grown on iso-C_5, 31 mol% when grown on an equal mixture of iso-C_5 and 33 mol% when grown on anteiso-C_5.

The fraction of glucolipid X constitutes approx. 15 mol% of the *A. laidlawii* membrane lipids when these contain 70 mol% of palmitoyl chains, the rest being oleoyl chains [26,27]. However, when the organism was fed with the saturated branched-chain fatty acids this lipid made up merely 1–4 mol% of the membrane lipids.

Acyl chain composition of membrane lipids

Addition of pantethein to the growth medium was necessary for the synthesis of long-chain branched fatty acids from short-chain precursors (see above). Acetate serves as elongation unit for the fatty acid synthesis in *A. laidlawii* [17], and [^{3}H]acetate was incorporated into the branched fatty acids when added to the medium. 90–95% of the radioactivity present in the membrane lipids was confined to the acyl chains.

The branched acyl chains constituted 80–87 mol% of the total amount of acyl chains when the organism was grown on the branched-chain fatty acid precursors. Straight saturated acyl chains made up the remaining fraction, with palmitoyl chains being the predominant ones (Table II). The precursors were incorporated into the long-chain fatty acids without prior metabolic conversion of the hydrocarbon chain. The average chain length was influenced by the structure of the precursor incorporated into the lipids. At 37°C the chain length parameter $(C_{17} + C_{19})/(C_{13} + C_{15})$ was decreased from 1.11 to 0.71 when anteiso-C_5 was replaced by iso-C_5 in the growth medium (Table II). A lower value of the chain length parameter was observed at 27°C as compared to 37°C with all supplements. Moreover, the ratio iso/anteiso acyl chains was decreased by decreasing the growth temperature.

A very effective incorporation of the long-chain branched fatty acids into the membrane lipids was achieved without using inhibitors [30,31] of the de novo fatty acid synthesis, and these acyl chains constituted between 94 and 98 mol% of the total amount of acyl chains. The fatty acids were neither elongated nor degraded to shorter chain lengths as judged by the GLC analyses. When 0.20 mCi/l of sodium [^{3}H]acetate was added to the medium, no radioactivity was incorporated into the acyl chains.

In some experiments *A. laidlawii* strain A was fed with different combinations of two long-chain branched fatty acids in order to investigate in what proportions these acids were incorporated into different membrane lipids (Table III). The acyl chain ratios in these lipids exhibited great variations, and two patterns of incorporation could be discerned: (1) one pattern with supplements containing iso-C_{17}; this acid is less efficient than the other acids in supporting growth of *A. laidlawii* strain A; (2) a second pattern with supplements containing fatty acids which support good growth when supplied alone. When iso-C_{17} was present in the medium, incorporation of this acid into glucolipid X was extremely high as compared to an-

TABLE II

ACYL CHAIN COMPOSITION OF TOTAL MEMBRANE LIPID EXTRACTS FROM *ACHOLEPLASMA LAIDLAWII* A GROWN ON BRANCHED-CHAIN FATTY ACID PRECURSORS

Pantetheine was added at a concentration of 12 μM (see text for explanation), the fatty acid precursors at 1.0 mM when supplied alone, and at 0.5 mM each when supplied together. Cells were grown at 27 and 37°C. n, normal; i, iso; a, anteiso; C_n, number of carbon atoms.

Acyl chain	Fatty acid precursor, growth temperature					
	a-C_5, 37°C	a-C_5, 27°C	a-C_5/i-C_5 37°C	a-C_5/i-C_5, 27°C	i-C_5, 37°C	i-C_5, 27°C
n-C_{12}	0.2	0.1	0.2	0.1	0.3	0.2
i-C_{13}	–	–	1.9	1.5	7.2	6.8
a-C_{13}	0.8	0.9	0.6	0.8	–	–
n-C_{14}	1.0	1.2	1.1	1.3	1.6	2.3
i-C_{15}	–	–	17.9	17.7	42.8	44.4
a-C_{15}	39.5	42.0	24.3	27.7	–	–
n-C_{15}	0.6	0.7	0.5	0.5	0.5	0.6
n-C_{16}	9.7	12.0	9.3	10.6	9.4	12.6
i-C_{17}	–	–	24.6	20.9	35.5	29.2
a-C_{17}	42.1	37.9	15.7	16.2	–	–
n-C_{17}	0.8	0.5	0.4	0.3	0.4	0.4
n-C_{18}	2.7	2.9	3.0	2.4	2.3	2.4
a-C_{19}	2.6	1.8	0.5	0.4	–	–
Acyl chain ratios						
$(C_{17}+C_{19})/(C_{13}+C_{15})$ [a]	1.11	0.93	0.91	0.79	0.71	0.57
Iso/anteiso [b]	–	–	1.08	0.89	–	–

[a] Molar ratio of branched acyl chains.
[b] Molar ratio iso/anteiso acyl chains.

teiso-C_{17} and iso-C_{15} (Table III). Next after glucolipid X, iso-C_{17} was preferentially directed into PG an MGDG. If anteiso-C_{15} was supplied together with anteiso-C_{17} or iso-C_{15}, incorporation of the C_{17} acid or the iso acid into glucolipid X was within the range found in the other lipids. Anteiso-C_{17} and iso-C_{15} were preferentially directed into GPDGDG and DGDG. It is noteworthy that a chain length difference of just two methylene groups in homologous fatty acids causes considerable differences in the acyl chain ratio in the various membrane lipids.

The temperature dependence of the incorporation of long-chain iso acids in relation to anteiso acids was also investigated. *A. laidlawii* was grown in a medium containing equimolar concentrations of anteiso-C_{15}, anteiso-C_{17}, iso-C_{15} and iso-C_{17}. The ratio iso/anteiso acyl chains was decreased from 1.07 to 0.68 when the growth temperature was changed from 37 to 27°C.

TABLE III

ACYL CHAIN RATIOS IN THE POLAR MEMBRANE LIPIDS FROM *ACHOLEPLASMA LAIDLAWII* A GROWN ON LONG-CHAIN BRANCHED FATTY ACIDS SUPPLIED IN PAIRS

The total concentration of fatty acids in the growth medium was 120 μM, and the fatty acids were supplied in equimolar concentrations. Cells were grown at 37°C. The acyl chain ratios are normalized, i.e., the value obtained for each lipid from the GLC analysis has been divided by the average value obtained for the total lipid extract. See Table I for abbreviations.

Lipid	Molar acyl chain ratios			
	a-C_{17}/a-C_{15}	i-C_{15}/a-C_{15}	i-C_{17}/a-C_{17}	i-C_{17}/i-C_{15}
GPDGDG	1.40	1.66	0.40	0.57
GPMGDG	0.76	0.93	0.58	0.37
PG	0.90	0.50	1.27	2.12
DGDG	1.17	1.28	0.60	0.74
MGDG	0.83	0.96	1.23	0.93
Glucolipid X	1.37	0.67	6.1	21.4

Discussion

Fatty acid growth requirement of A. laidlawii

It has been claimed that *A. laidlawii* strain A has an absolute growth requirement for a long-chain unsaturated or cylcopropane fatty acid [18–21]. However, it is shown here that this strain grows equally well on certain short-chain and long-chain iso and anteiso fatty acids. Henrikson and Panos [32] have stated that it is highly unlikely that the B strain has an absolute growth requirement for an unsaturated fatty acid. However, the content of unsaturated acyl chains in the membrane lipids was between 5 and 8% in their experiments. Such a low fraction of unsaturated fatty acids, the rest being saturated ones, is able to support growth of the A strain as well [22]. The saturated fatty acids n-C_{12} and n-C_{14} to n-C_{18} are all unable to promote growth of either the A strain or the B strain in the medium used by us [22,23,33]. Thus, no difference between these strains, as regards their fatty acid growth requirements, appears to exist. The resemblance between the strains has also been suggested by Rodwell and Mitchell [34].

Regulation of polar head group composition

In *A. laidlawii* membranes the molar ratio MGDG/DGDG is reduced, and the molar ratio ionic/non-ionic lipids is raised, when the ratio saturated/*cis*-unsaturated straight acyl chains in the lipids is decreased [7,9]. These regulation mechanisms have been explained on the basis of lipid molecular geometry [4,7,10]. An increase in the fraction of *cis*-unsaturated acyl chains will increase the hydrophobic bulkiness of lipid molecules, leading to more wedge-like molecular shapes and enhanced tendencies to form non-lamellar phases [4,13,14]. A stable lamellar phase is restored by the organism by lowering the ratio between lipids forming non-lamellar phases and lipids forming lamellar phases. *A. laidlawii* achieves this by making use of the regulation mechanisms described above, since MGDG forms a reversed hexagonal phase while DGDG and the ionic lipids form a lamellar phase [13,29]. The theory of self-assembly of amphiphiles [11,12], on which this discussion is based, is applicable when the organism incorporates iso and anteiso fatty acids as well. A PE molecule becomes more wedge-shaped when the ratio iso/anteiso acyl chains in this lipid is decreased [3]. The shape of lipid molecules with other polar head groups must be affected in the same way by this acyl chain ratio, since it has been shown that a certain structural change in the acyl chains causes similar changes in the phase equilibria of several lipid-water systems [4]. Consequently the ratio MGDG/DGDG can be predicted to decrease and the ratio ionic/non-ionic lipids predicted to increase when anteiso acyl chains replace iso acyl chains in the *A. laidlawii* membrane lipids. These predictions were born out. Moreover, *A. laidlawii* strain B regulates the MGDG/DGDG ratio in the predicted manner when iso and anteiso fatty acids are incorporated into its membrane lipids [35]. The MGDG/DGDG ratio in membranes of *A. laidlawii* strain A enriched with oleic acid is lower than the ratio obtained when the organism is grown on anteiso acids [7]. This finding can also be interpreted in terms of lipid molecular geometry. Dioleoylphosphatidylethanolamine has a more pronounced wedge-shape than PE containing primarily anteiso acyl chains, since the former lipid species forms a reversed hexagonal phase in excess water at room temperature [36], while the latter species forms a lamellar phase under the same conditions [3]. Thus the difference in packing properties between iso, anteiso and *cis*-monounsaturated fatty acids is reflected by the variation in the MGDG/DGDG ratio in membranes of *A. laidlawii* strain A.

The molar ratio MGDG/DGDG is decreased when the incorporated acyl chains are elongated from 15 to 17 carbon atoms (Table I). This regulation has been observed to occur in *A. laidlawii* strain B as well [35]. Moreover, this lipid ratio is reduced when *A. laidlawii* strain A is grown on oleic acid instead of palmitoleic acid (Ref. 22; and L. Rilfors and Å. Wieslander, unpublished results). It has been noted that elongation of the hydrocarbon chains increases the tendencies for glycerolipids to form cubic and reversed hexagonal phases [4,37,38]. A reduction of the ratio MGDG/DGDG thus compensates for the change in packing properties caused by the acyl chain elongation.

Regulation of acyl chain composition

The temperature-induced regulation of the ratio iso/anteiso acyl chains in the membrane lipids of *A. laidlawii* strain A can be explained by the difference in packing properties between these acyl chains. An increased temperature favours the formation of cubic or reversed hexagonal phases [4]. The organism responds by increasing the ratio iso/anteiso acyl chains in the lipids, which supresses the tendencies to form these phases [3]. The temperature-dependent regulation of the ratio iso/anteiso acyl chains in biological membranes is parallelled by the regulation of the ratio saturated/*cis*-monounsaturated straight acyl chains [7].

The average acyl chain length in the membrane lipids from *A. laidlawii* strain A was affected by the growth temperature as well as by the structure of the branched fatty acid precursor fed to the cells (Table II). The average chain length of the de novo synthesized saturated fatty acids is temperature-invariant in *A. laidlawii* strain B [6]. However, an increase in the chain length when the growth temperature is raised has been observed to occur in many other organisms, those containing branched-chain fatty acids [5,39] as well as those containing straight-chain fatty acids [40]. When *A. laidlawii* strain A was grown on iso-C_5 the average length of the de novo synthesized fatty acids was less than when the organism was grown on anteiso-C_5. This finding is supported by results obtained with the B strain [41]. The incorporation of straight-chain saturated, iso, anteiso and straight-chain *cis*-monounsaturated fatty acids into the membrane lipids as a function of fatty acid chain length was investigated; these acids made up a maximum fraction of the lipid acyl chains when their chain lengths were C_{15}, C_{16}, C_{17} and C_{18}, respectively. Furthermore it was shown that a mutant of *Escherichia coli* synthesizing only straight-chain saturated fatty acids contained membrane lipids with an average acyl chain length of C_{14} while the average length in the wild-type membrane lipids (40 mol% saturated acyl chains) was approximately C_{16} [42]. However, the described regulations of the acyl chain length probably cannot be explained in terms of lipid molecular geometry. An increase in the temperature and a reduction of the iso/anteiso and straight-chain saturated/*cis*-unsaturated acyl chain ratios will increase the tendencies of lipid-water systems to form cubic and reversed hexagonal phases [4]. By increasing the acyl chain length as a response to the above-mentioned alterations the phase equilibria of lipid-water systems will be further shifted towards the non-lamellar phases [4,37,38]. However, lipid molecular geometry is not the only physicochemical property that has to be regulated, and it is possible that the aim of the chain length regulation is a regulation of the lipid bilayer thickness. An increase in the temperature and a reduction of the above-mentioned acyl chain ratios reduce the order parameter of the chain segments [43–46]. This will lead to a decrease in the average acyl chain length, since there is a direct relationsip between this quantity and the order parameter of the chain segments [43,47]. The decrease in the acyl chain length in turn results in a bilayer thinning [48,49]. Be elongating the acyl chains the organisms thus compensate for this effect. A regulation of the bilayer thickness may be of importance for living cells, since the activity of several trans-membrane enzymes and transport proteins is dependent on the length of the surrounding acyl chains [48,50–53]. Moreover, the permeability of both liposomal membranes and *A. laidlawii* membranes for glycerol and erythritol is altered when the membrane lipids contain acyl chains of varying length [54,55]. Finally, the possibility also exists that the aim of the chain-length regulations is a regulation of the gel to liquid-crystalline transition temperature interval in relation to the growth temperature, which might affect enzyme activities and the membrane permeability [6].

Acyl chain composition in individual lipids

When *A. laidlawii* strain A was grown in iso-C_{17} together with iso-C_{15} or anteiso-C_{17} these acids were incorporated in varying proportions into the individual membrane lipids (Table III); the ratios iso-C_{17}/iso-C_{15} and iso-C_{17}/anteiso-C_{17} were higher than the average in glucolipid X, PG and MGDG. When strains A and B are fed with palmitic and oleic acid the former acid is preferentially incorporated into glucolipid X and MGDG [22,27]. Iso-C_{17} and palmitic acid have the highest melting points in the three supplements discussed, and they support poor growth and no growth at

all, respectively, of *A. laidlawii*. The acyl chain composition in the individual membrane lipid is thus dependent on the physicochemical properties of the chains. Glucolipid X incorporates extreme amounts of iso-C_{17}, which supports the suggestion put forward earlier [23] that this species acts as a scavenger lipid withdrawing diacylglycerol with extreme acyl chain compositions. It can be concluded that each one of the lipid-synthesizing enzymes in *A. laidlawii* selects a proper substrate, which results in the maintenance of a carefully controlled acyl chain composition of the individual lipid species.

Properties of branched-chain fatty acids

The straight-chain saturated and unsaturated fatty acids have been payed the greatest attention in physiological as well as physicochemical studies of biological membranes. However, sufficient data concerning the properties of iso and anteiso fatty acids are now available to allow a comparison to be made between these fatty acids and the straight-chain fatty acids. Such a comparison is of biological relevance, since straight saturated and unsaturated acyl chains on the one hand, and iso and anteiso acyl chains on the other, often occur together in large amounts in biomembranes. In the summary presented below a straight-chain saturated fatty acid is compared to a straight-chain *cis*-monounsaturated fatty acid, and an iso fatty acid is compared to an anteiso fatty acid.

(1) Straight-chain saturated and iso fatty acids per se [2], and synthetic phosphatidylcholines in the condensed state containing these acids [56], occupy smaller areas at the air-water interface. However, synthetic phosphatidylcholines in the expanded state containing straight-chain saturated, iso, anteiso, and straight-chain *cis*-monounsaturated acids, all occupy approximately the same area [56].

(2) Straight-chain saturated and iso fatty acids have higher melting points [1,57], and synthetic phosphatidylcholines containing these acids have higher gel to liquid-crystalline transition temperatures [58–61].

(3) Straight-chain saturated and iso fatty acids are preferentially incorporated into position 1 of glycerolipids [62,63].

(4) The lamellar phase of PE is more stable when the lipid contains straight-chain saturated acyl chains [36–38], and is enriched in iso acyl chains [3].

(5) Straight-chain saturated and iso fatty acids incorporated into lipids present in a biological membrane have higher order parameters [44,46].

(6) The molar fraction of straight-chain saturated and iso acyl chains in biological membrane lipids increases when the growth temperature is increased (Table II) [4,5].

(7) Incorporation of straight-chain saturated and iso fatty acids into membranes lipids is accompanied by a reduction of the average acyl chain length (Table II) [41,42].

(8) Incorporation of larger relative amounts of straight-chain saturated and iso fatty acids into *A. laidlawii* membrane lipids results in the same qualitative changes in polar head group composition (Table I) [7].

It is clear that membrane lipids containing iso and anteiso acyl chains have distinctively different physicochemical properties. The two kinds of acyl chains have different packing properties in a biological membrane, and are able to fulfil roles similar to those played by straight-chain saturated and *cis*-monosaturated acids, respectively. However, the difference in physicochemical properties between iso and anteiso fatty acids is less than the difference between straight-chain saturated and *cis*-monounsaturated fatty acids. If fatty acids of the same chain length are considered, the four types of acids can be arranged in the following order with respect to physicochemical properties: straight-chain saturated, iso, anteiso and straight-chain *cis*-monounsaturated fatty acids.

The theory of self-assembly of amphiphiles [11,12] is used to interpret the lipid regulation mechanisms operating in *A. laidlawii* when the organism incorporates straight-chain as well as branched-chain fatty acids. Changes in the structure of the hydrophobic region, brought about in two different ways, result in directly correlated changes in the polar head group structure. This finding supports our earlier statement [4,7,10] that the effective shape of the lipid molecules is one important factor governing the regulation of the lipid composition in *A. laidlawii* membranes.

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