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Membrane potential, lipid regulation and adenylate energy charge in acyl chain modified *Acholeplasma laidlawii*

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In *Acholeplasma laidlawii* variations induced in the transmembrane electrical potential have been shown to affect the membrane lipid composition. Particularly the molar ratio between the predominant glucolipids, monoglucosyldiacylglycerol and diglucosyldiacylglycerol, decreases upon hyperpolarization and increases upon depolarization (Clementz et al. (1986) Biochemistry 25, 823–830). Upon variation of the degree of membrane fatty acyl chain unsaturation, known to affect the passive permeability for a number of small molecules, there was no significant correlation between acyl chain composition and the magnitude of the electrical potential. Hyperpolarization by valinomycin decreased the glucolipid ratio for all kinds of membranes, but the size of the decrease was not correlated to the acyl chain composition. However, a clear relationship, independent of acyl chain composition, was found between the extent of hyperpolarization and the size of the decrease in the glucolipid ratio. The adenylate energy charge value (E_c) of the cells was affected by the acyl chain composition, although not exclusively by the proportion of unsaturation. Furthermore, a larger hyperpolarization upon valinomycin addition was accompanied by a stronger reduction in E_c.

Introduction

The insulating properties of biological membranes are of crucial importance for the maintainance of electrical and chemical gradients across the membranes, and thus for the storage of chemiosmotic energy. Any factor, internal or environmental, that affects these gradients, e.g. changes in membrane permeability, is likely to affect the energy costs involved in maintaining the necessary gradients. The passive permeability is to a large extent determined by the properties of the membrane lipids. Factors that decrease the molecular ordering, e.g., increased acyl chain unsaturation, increased temperature or the absence of cholesterol, tend to increase the passive permeability of lipid bilayers for a number of molecules [1].

Acholeplasma laidlawii is a simple bacterium whick lacks a cell wall; it grows at alkaline pH, lacks cytochromes and obtains ATP from substrate level phosphorylation (fermentation of glucose to lactate) [2]. Part of the cell energy is used to accumulate potassium against a concentration gradient by an energy-dependent process [3,4] and to keep a low intracellular concentration of sodium [5]. Most likely the transmembrane potential is created by hydrolysis of ATP. In *A. laidlawii* containing 95% oleoyl chains in the membrane

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lipids, a transmembrane electrical potential of −50 mV (inside negative) but no ΔpH was found [6].

A. laidlawii is unable to synthesize unsaturated fatty acids and the endogenous fatty acid synthesis can be repressed [7,8]. This offers almost unique possibilities to control the acyl chain composition of the membrane lipids by addition of selected fatty acids to the growth medium [7,9,10]. For acyl chain modified *A. laidlawii* cells, or liposomes derived from their membrane lipids, the passive permeability for nonelectrolytes, such as glycerol and erythritol, increases with the extent of acyl chain unsaturation [11–13] in a manner very similar to what has been observed for synthetic lipids [11]. It can be envisaged that $\Delta\psi$ or the cell energy costs should be different in cells modified with various acyl chains.

In cells containing 95% oleoyl chains, hyper- or depolarization of $\Delta\psi$ with low concentrations of valinomycin or tetraphenylphosphonium (TPP^+) causes a decrease or an increase, respectively, in the molar ratio between the two predominant polar membrane lipids, monoglucosyldiacylglycerol (MGDG) and diglucosyldiacylglycerol (DGDG) [6]. These lipids form reversed hexagonal and lamellar phases with water, respectively. The MGDG/DGDG ratio governs the phase equilibria (lamellar or non-lamellar phases) of the entire polar lipid mixture found in the membrane [14]. Non-lamellar phases lack the insulating properties of bilayer membranes. Changes in the MGDG/DGDG ratio occur as a response to any disturbance that upsets the bilayer stability of the membrane, e.g., variations in temperature, acyl chain composition, cholesterol content [9], or the presence of foreign molecules such as alcohols and hydrocarbons [15] or sterols [16]. This response in glycolipid ratio aims to maintain an optimal packing stability of the membrane [17,18].

Material and Methods

Growth conditions. Acholeplasma laidlawii B(ju) was grown in a lipid-depleted bovine serum albumin-tryptose medium [9]. The same batch of growth medium was used throughout all experiments to minimize the effects of medium composition other than those experimentally inflicted. The growth medium was supplemented with various combinations of palmitic (16 : 0) and oleic ($18:1_c$) acids, or with linoleic ($18:2_c$) acid, to a total concentration of 150 μM. Membrane lipids were radioactively labelled by the addition of ^{14}C-labelled fatty acids to a total activity of 10 μCi/l. The ^{14}C-isotopes were added to give equal specific activities of the fatty acids present in the growth medium. All the experiments were conducted during shift procedures. Cells were grown at 30 °C to a turbidity of approximately 0.3 at 540 nm. At this time ($t = 0$) the culture was divided into two equal parts, which were supplemented with valinomycin (10 nM) or ethanol (as a control), respectively. The cultures were then incubated for 15 min or 6 h. The stock solution of valinomycin was dissolved in ethanol (99.5% v/v). The addition of ethanolic solutions to the growth medium never exceeded 0.5% (v/v).

Lipid analysis. The cells were harvested by centrifugation at $32\,000 \times g$ for 8 min at 5 °C and washed once in β-buffer [9]. Membranes were prepared by osmotic lysis and washed twice in β-buffer diluted 1/20 (v/v). Lipids were extracted with chloroform/methanol (2 : 1, v/v), purified by Sephadex G-25 (Pharmacia, Sweden) chromatography, separated by thin-layer chromatography, and quantified by liquid scintillation counting as described [9]. Gas-liquid chromatography was used to analyze the total fatty acyl chain composition of the membranes [7].

Measurement of $\Delta\psi$. The transmembrane electrical potential was determined by measuring the accumulation of the lipophilic cation [^{3}H]TPP^+. The intracellular concentration of the probe was determined after centrifugation of the cells through silicon oil and each sample was corrected for the actual amount of cells pelleted after centrifugation [6]. Measurement of intracellular volume, for calculation of intracellular concentrations, was made for each individual fatty acid supplementation [6].

Measurement of adenylate enery charge (E_c). 100 μl samples from growing cultures, with and without valinomycin, were withdrawn at 15 min and 6 h after the shift. The samples were diluted in 30 volumes of extraction buffer (0.1 M Tris acetate/2 mM EDTA/1% (w/v) trichloroacetic acid (pH 7.75)), vortexed and immediately frozen at below −70 °C. The samples were thawed just

before analysis and 100 μl was distributed to each of three measuring tubes and were further diluted 10-times in measuring buffer (0.1 M Tris acetate/5.5 mM magnesium acetate/14.5 mM potassium acetate, 0.1 mM phospho*enol*pyruvate (pH 7.75)). The three tubes were used for measurement of the amount of the individual nucleotides ATP, ADP, and AMP, respectively. Triplet measurements were made for each individual nucleotide. ATP was measured by the use of a luciferin/luciferase assay (ATP assay kit, LKB Wallac, Sweden) and a luminometer (Bioluminat LB9500, Berthold, F.R.G.). ADP was measured as ATP after enzymatic conversion by pyruvate kinase (20 U/ml) (Sigma). AMP was measured after conversion to ATP by adenylate kinase (50 U/ml) (Sigma) and pyruvate kinase (20 U/ml). For conversion, the samples were incubated 25 min at room temperature. Each sample was calibrated internally by measurement of the sample before and after addition of a known amount of ATP. Adenylate energy charge (E_c) was calculated [19] by

$$E_c = ([ATP] + \tfrac{1}{2}[ADP])/([ATP] + [ADP] + [AMP])$$

Determination of glucose consumption. 2 ml samples from growing cultures, with and without valinomycin, were withdrawn 15 min and 6 h after the shift. These samples were immediately frozen at −20°C. Before analysis, the samples were deproteinized with 2% (w/v) trichloroacetic acid. After 30 min incubation at room temperature, the samples were centrifuged in a desk top centrifuge (Wifug, Doctor) at 3700 rpm for 5 min to sediment the precipitate. The samples were then diluted 10-times to decrease the trichloroacetic acid concentration. Glucose was determined by a colorimetric glucose oxidase/peroxidase test (Sigma, U.S.A.). Five measurements were made for each sample.

Results

Acyl chain modification of membrane lipids

The growth medium was supplemented with different combinations of fatty acids to obtain cell membranes with different physical properties, especially with regard to molecular order and passive permeabilities. This yielded membranes with acyl chain compositions resembling the composition of the fatty acids added to the growth medium (see Table I). The change in acyl chain composition in cells supplemented with various combinations of 16 : 0 and 18 : 1 results in membranes with gel to liquid-crystalline phase transition midpoints ranging from approx. 13°C down to −4°C and a transition width of approximately 20 Cdeg [14]. Cells with 18 : 2 show a phase transition midpoint of −19°C and a width of approx. 20 Cdeg [20].

The degree of membrane acyl chain unsaturation influenced the lipid composition in a manner consistent with earlier observations [7]. An increase in the proportion of unsaturated acyl chains in the membrane induced a decrease in the MGDG/DGDG ratio, see Fig. 2A (control cells). Furthermore, the proportion of charged lipids increased from 26% to 34% (mol/mol), data not shown.

Membrane potential and lipid regulation in modified cells

A transmembrane electrical potential between −52 to −62 mV was recorded for the differently modified control cells, see Fig. 1A. The absolute value of $\Delta\psi$ was not directly correlated to the proportion of unsaturated acyl chains in the membrane. The highest value of $\Delta\psi$ (−62 mV) was obtained for cells containing the lowest propor-

TABLE I

ACYL CHAIN COMPOSITION IN *A. LAIDLAWII*

The organisms were grown at 30°C to a turbidity of approximately 0.5 at 540 nm. The acyl chain composition was analyzed by gas-liquid chromatography.

Supplementation to growth medium	Acyl chain composition in the membrane (mol/100 mol)		
	16:0	18:1$_c$	18:2
16:0/18:1$_c$ (μM/μM)			
120/30	62	32	–
75/75	45	52	–
30/120	17	82	–
0/150	9	91	–
18:2 (μM)			
150	9	2	85

tion of unsaturation, i.e. 32% oleic acid. No significant variation was found between cells with a higher degree of unsaturation (see Fig. 1A). When

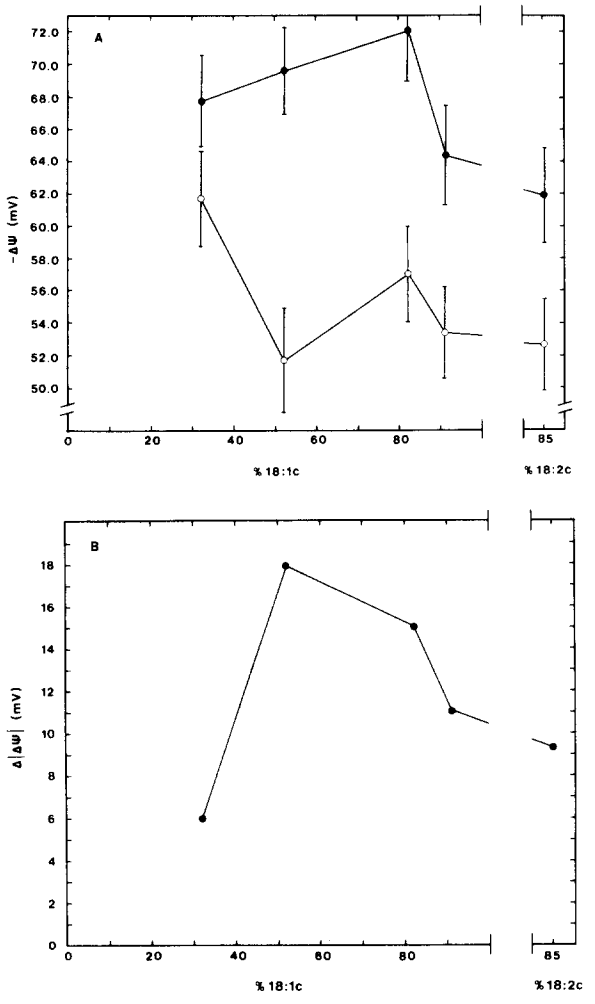

Fig. 1. Transmembrane electrical potential ($\Delta\psi$) in acyl chain modified cells of *A. laidlawii*. Cultures were grown in the presence of different combinations of palmitic and oleic acids, or linoleic acid. The cultures were divided in two parts, one of which was supplemented with 10 nM of the K^+-ionophore valinomycin whereas the other part served as a control. The $\Delta\psi$ was determined 6 h after the shift. %18:1 = molpercentage of oleoyl chains of the total acyl chains in the membrane. %18:2 = molpercentage of linoleoyl chains of the total acyl chains in the membrane. Bars represent 90%-confidence limits for the combined error in TPP^+-accumulation and intracellular water space. The 90%-confidence limits for the TPP^+-accumulation alone are 1.5–2.0 mV. (A) $\Delta\psi$ in different cells with (●) and without (○) valinomycin. (B) Increase in $\Delta\psi$ in valinomycin-treated cells, as compared to control cells ($\Delta|\Delta\psi|$).

valinomycin, a K^+-ionophore, was added to the growing cultures, a hyperpolarization was observed in all types of modified cells (Fig. 1A). This hyperpolarization was evident already 15 min after valinomycin addition (data not shown). There was a pronounced difference in hyperpolarization between cultures with different acyl chain composition. This hyperpolarization, $\Delta(\Delta\psi)$, as well as the differences observed, lasted for at least 6 h (Fig. 1B) during which time the cells continued to grow, albeit at reduced growth rates. In this connection it should be mentioned that in oleoyl-enriched cells, addition of valinomycin is accompanied by a drastic reduction in the intracellular K^+-concentration from 190 mM to 45 mM in less than 60 s [6]. The concentration stabilizes at 25–30 mM during the 90 min following the valinomycin addition. These intracellular levels should be compared to the extracellular concentration of 3.5–4.0 mM in the growth medium [6]. It seems reasonable to assume that this K^+-loss also occurs in the other types of acyl chain-modified cells. The increase in $\Delta\psi$ observed upon valinomycin addition was accompanied by a significant decrease in the MGDG/DGDG ratio. This was true for all types of fatty acid supplementations (see Fig. 2A). However, the decrease in the MGDG/DGDG ratio, compared to the control cells, Δ(MGDG/DGDG), depended on the acyl chain composition in a complex manner (Fig. 2B). There was a similar dependence of Δ(MGDG/DGDG) and $\Delta(\Delta\psi)$ on the acyl chain composition (Figs. 1B, 2B). A larger hyperpolarization resulted in a proportionally stronger reduction in the MGDG/DGDG ratio for the different palmitic/oleic acid supplemented cells as shown in Fig. 3. Linoleoyl-enriched cells did not follow the same proportionality.

Energy charge and membrane yields

As $\Delta\psi$ is connected to cell energy metabolism, adenylate energy charge (E_c) was chosen as a measurement of the energetic status of the cells. The E_c increased with an increased proportion of oleoyl chains in the membranes. The lowest value of E_c was found in cells supplemented with linoleic acid (Table II). E_c fell only slightly in the control culture during the 6 h incubation after shift. However, valinomycin caused a significant

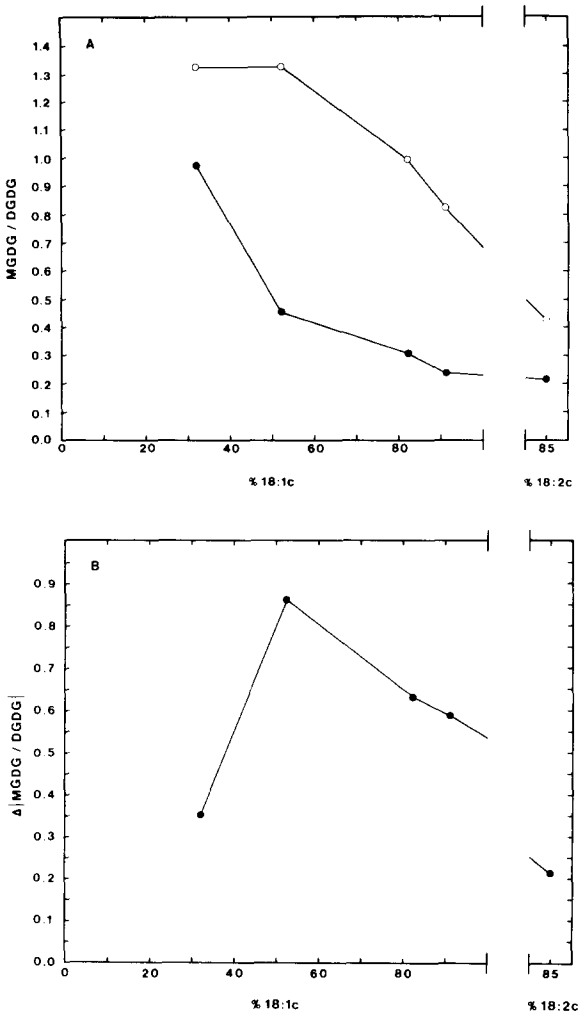

Fig. 2. Monoglucosyldiacylglycerol/diglucosyldiacylglycerol ratio (MGDG/DGDG) in acyl chain modified cells of *A. laidlawii*. The experimental procedures were exactly the same as described in Fig. 1. (A) MGDG/DGDG ratios in control cells (○) and in valinomycin-treated cells (●). (B) Relative decrease of MGDG/DGDG in valinomycin-treated cells as compared to control cells (Δ|MGDG/DGDG|).

decrease in energy charge already 15 min after addition. The size of the decrease in E_c was different with different fatty acid supplementations. After a further 6 h incubation with valinomycin, E_c had fallen in most cases to a value as low as approx. 0.45, (see Table II). In the adenylate pool a relative decrease in ATP was most pronounced after 15 min whereas the further decrease in E_c after 6 h was mainly attributed to a

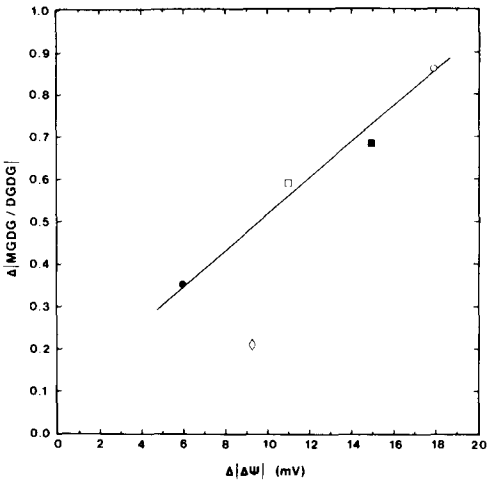

Fig. 3. The effect of hyperpolarization ($\Delta|\Delta\psi|$) on the decrease in glucolipid ratio (Δ|MGDG/DGDG|). See Fig. 1 for experimental procedures. The cultures were supplemented with 120/30 (●); 75/75 (○); 30/120 (■); 0/150 (□) μM palmitic/oleic acid, or with 150 μM linoleic acid (◇).

relative increase in AMP (data not shown). Fig. 4 shows that the difference in E_c between control cells and valinomycin-treated cells (ΔE_c) became larger as the size of the hyperpolarization increased.

TABLE II

THE EFFECT OF VALINOMYCIN ON ADENYLATE ENERGY CHARGE (E_c) IN CELLS OF *A. LAIDLAWII* WITH DIFFERENT ACYL CHAIN COMPOSITIONS

E_c was determined 15 min and 6 h after addition of valinomycin (10 nM) or ethanol (as control). See Table I for acyl chain composition of cells grown with different fatty acid supplements. Error limits as 90%-confidence limits, 0.025–0.035.

Fatty acid supplements	Adenylate energy charge (E_c)			
	control		+ valinomycin (10 nM)	
	15 min	6 h	15 min	6 h
16:0/18:1$_c$ (μM/μM)				
120/30	0.64	0.59	0.55	0.44
75/75	0.70	0.65	0.53	0.43
30/120	0.72	0.64	0.57	0.45
0/150	0.78	0.70	0.64	0.52
18:2 (μM)				
150	0.59	0.51	0.57	0.47

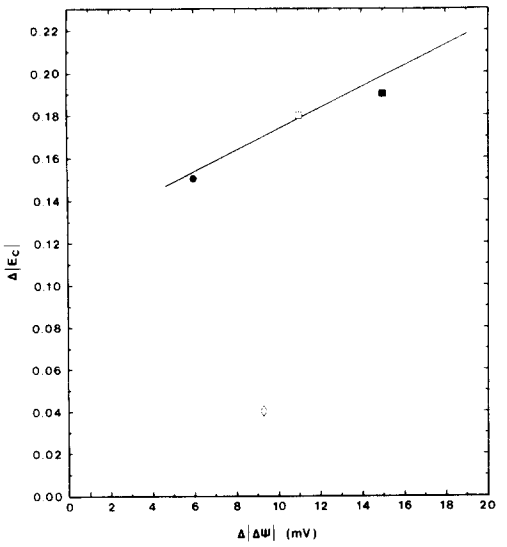

Fig. 4. The depletion of adenylate energy charge ($\Delta \mid E_c \mid$) as a function of hyperpolarization ($\Delta \mid \Delta\psi \mid$). E_c = ([ATP] + $\frac{1}{2}$[ADP])/([ATP]+[ADP]+[AMP]). See Fig. 1 for experimental procedures. Symbols as in Fig. 3. Error limits for E_c as 90%-confidence limits, 0.025–0.035.

TABLE III

MEMBRANE YIELDS AFTER ADDITION OF VALINOMYCIN

Membrane yields were determined 6 h after addition of valinomycin (10 nM) or ethanol (as control). Membrane amounts were estimated from the amounts of radioactivity labelled polar membrane lipids. Lipids constitute 40–50% (w/w) of membrane amounts.

Fatty acid supplements	Mol polar membrane lipids produced per mol glucose consumed	
	control	+ valinomycin (10 nM)
16:0/18:1$_c$ (μM/μM)		
120/30	5.0	2.7
75/75	5.1	2.0
30/120	6.3	3.4
0/150	5.1	1.3
18:2 (μM)		
150	4.5	0.8

Another parameter that gives information about energy requirements is the amount of glucose consumed during the 6 h incubation following shift. During this incubation period control cells consumed between 3.5 to 6.5 mmol glucose per liter culture whereas, for cells incubated with valinomycin, 1.7 to 4.2 mmol glucose per liter culture was consumed. At no time was there any shrotage of glucose in the growth media (the initial concentration was 40 mM). Merely glucose consumption is not a sufficient measure of the cellular energy utilization. The consumption has to be combined with the cell growth and the result expressed as a cell yield coefficient. Membrane yield was chosen as a representative for the cell yield coefficient as no further manipulation of the cells were necessary to obtain membrane lipid amounts. We believe that membrane yield coefficients are adequate for qualitative comparisons. Even though the amount of glucose consumed varied among the control cultures, membrane yield coefficients were fairly similar between these cultures (Table III). However, addition of valinomycin, which was accompanied by a hyperpolarization and a decrease in E_c, decreased the membrane yield coefficients significantly (see Table III). This was not due to large differences between the cultures in the amount of membrane lipids synthesized following valinomycin addition, but mainly due to increased utilization of glucose for other purposes than synthesis of cell material. Among these cultures the decrease in yield coefficient was most pronounced for cells with a higher proportion of unsaturated acyl chains.

Discussion

Changes in $\Delta\psi$ and lipid regulation

The observed decrease in the MGDG/DGDG ratio (Fig. 2A) in membranes with higher contents of unsaturated acyl chains can be explained by their destabilizing effect on the lamellar structure of the bilayer. To maintain a stable bilayer the proportion of MGDG is decreased and consequently a reduction is observed in the glucolipid ratio [14,18]. Similarly, the increase of charged lipids has been proposed to be a compensatory effect for the dilution of charges by the increased lateral surface areas of lipid unsaturated acyl chains compared with saturated ones. By increasing the percentage of charged lipids the mem-

brane surface charge density is maintained at a constant value [21].

Our understanding of the influence of lipid polar headgroup and acyl chain composition on bilayer permeability to ions and uncharged molecules is at present rather primitive (see Cullis et al. [1]). Nevertheless, it is well established that a decrease in the order of the acyl chains yields an increase in cation permeability in vitro [22,23]. Except for palmitate-enriched cells, there seems to be no dependence of the size of $\Delta\psi$ on membrane lipid unsaturation in *A. laidlawii,* Fig. 1A. This indicates that the cell can compensate for the established differences in permeability for these modified membranes. The size of $\Delta\psi$ thus seems to be primarily governed by other factors than acyl chain composition. The slightly higher value in palmitate-enriche cells may indicate a dependence on acyl chain order in cells with a high proportion of saturated acyl chains. A higher incorporation than 62% palmitoyl chains in the membrane lipids, see Table I, was unfortunately not found even though the growth medium was supplemented with a higher proportion of 16 : 0.

A hyperpolarization by valinomycin, previously observed in oleoyl-enriched cells [6], was found in all cells (Figs. 1A, 1B). Here too a direct dependence of the increase in $\Delta\psi$ on acyl chain unsaturation might have been expected but seems not to occur (Fig. 1B). This is perhaps not surprising considering the strongly increased complexity of a biological membrane compared with a simple lipid bilayer. Not only passive permeability of ions through the bilayer but also carrier-mediated ion permeability may be affected by the lipid composition in *A. laidlawii* B [24,5]. Also, the active transport of glucose is increased by an increased unsaturation of the membrane lipid acyl chains [25]. Thus several other factors that are involved in the creation of the final steady-state value of $\Delta\psi$ may be affected by changes in acyl chain composition. However, in cells supplemented with different proportions of 16 : 0 and 18 : 1*c*, a relationship between the change in $\Delta\psi$, i.e., the hyperpolarization, and the change in MGDG/DGDG ratio compared with control cells, was found, see Fig. 3. It should be noted that the magnitude of the change in glucolipid ratio upon hyperpolarization does not follow the extent of acyl chain unsaturation. This means that the effect is not directly dependent on acyl chain unsaturation per se. The fact that the change in MGDG/DGDG ratio correlates well with the size of hyperpolarization lends further support to our previous suggestions [6] that the transmembrane electrical potential, directly or indirectly, affects lipid organization and phase equilibria in the *A. laidlawii* membrane. We have previously established a constancy in the lamellar-non-lamellar phase equilibria of total polar lipid mixtures from different 16 : 0 plus $18:1_c$ enriched membranes, a constancy preserved by the accompanying changes in MGDG/DGDG ratios [14].

As can be seen from Fig. 3, the change in MGDG/DGDG ratio in 18 : 2-enriched cells does not correlate with the $16:0/18:1_c$ mixtures. For a similar hyperpolarization, the change in MGDG/DGDG ratio is significantly lower in 18 : 2-enriched cells than for $16:0/18:1_c$ mixtures. $18:2_c$ is the most unsaturated fatty acid that allows growth of *A. laidlawii*, but at lower cell yields than more saturated ones. The dynamic molecular shape of the membrane lipids with 18 : 2*c* chains results in a very low MGDG/DGDG ratio even without valinomycin, cf. Ref. 16 and Fig. 2A. We have previously observed that such cells, compared to more saturated ones, have a limited capacity to further lower the MGDG/DGDG ratio as a response to other perturbants such as different steroids or monovalent cations [16,21] (Wieslander, unpublished observations). There might be a lower limit of MGDG/DGDG amounts that is necessary for a functioning membrane. This is indicated by the plateau level for the MGDG/DGDG ratio with increasing acyl chain unsaturation in the presence of valinomycin (Fig. 2A). hence, the initially low ratio in $18:2_c$ cells cannot, upon hyperpolarization, be decreased as much as for the more saturated cells.

Acyl chain composition, $\Delta\psi$ and energy charge

Table II shows that there is a significant difference in E_c between palmitate- and oleate-enriched cells grown in the absence of valinomycin. Furthermore, linoleoyl-enriched cells had a much lower value in E_c. Most likely the reasons for these differences is equally complex as the factors governing the size of $\Delta\psi$. It is obvious, however,

that the adenylate energy charge can be influenced by the acyl chain composition. This emphasizes the fact that the membrane lipids are important in many different ways in the physiological status of *A. laidlawii* cells. The E_c decreased in control cells during the 6 h incubation. This is consistent with the findings of Beaman and Pollack [26,27] that E_c decreases when the cells enter the stationary phase.

The intracellular concentration of K^+ was decreased during the hyperpolarization of oleoyl cells [6]. Since the steady-state concentration following hyperpolarization (30 mM) is significantly higher than the growth medium concentration, a rapid uptake of K^+ must occur concomitantly. The rapid K^+ exchange observed previously [3] supports this notion. During the hyperpolarization caused by valinomycin, energy charge was decreased in all cells (Table II). We suggest that the decrease in energy charge is due to increased demands of ATP for maintaining a growth-supporting concentration of K^+ during conditions of enhanced efflux, i.e., valinomycin-mediated transport. This is supported by the results in Fig. 4. The size of the hyperpolarization can be assumed to be determined by the extent of K^+-efflux. If the energy costs for K^+-uptake increase with the size of hyperpolarization, it is reasonable to assume that E_c will decrease proportionally. The decrease in energy available for cell mass synthesis (Table III) is in line with this reasoning.

Membrane potential and lipid packing

The interaction between the transmembrane electrical potential and the mechanisms regulating the lipid composition can probably have several explanations. With the use of different ionophores we have earlier shown that there is no obvious correlation between the intracellular concentration of potassium and the glucolipid ratio [6]. Although our work cannot exclude that the observed effect on glucolipid ratio in response to valinomycin may be attributed to other factors, e.g. electrically neutral flow of other ions, one alternative explanation is that the potential affects the conformation of lipid acyl chains (kinks and *trans-gauche* isomerizations) which affect the packing properties and thus the phase equilibria of the membrane lipids, cf. [6]. Recently, conformational analyses of deuterated *A. laidlawii* lipid acyl chains indicated a difference in membrane acyl chain conformation in intact living cells compared to purified membranes [28]. According to the authors one plausible explanation to this is the presence of a transmembrane electrical potential in the living cell. We have previously shown that conformational and shape-related changes of membrane lipids are of upmost importance for the phase equilibria of these lipids [14,18]. It is therefore hardly surprising that the forces imposed on a membrane by the transmembrane electrical potential may have an effect on the lipid regulation in *A. laidlawii.*

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