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THE GLYCOLIPID FATTY ACIDS OF *PORPHYRIDIUM PURPUREUM* CULTURED IN THE PRESENCE OF DETERGENTS

HARRI NYBERG and KRISTIINA KOSKIMIES-SOININEN

Department of Botany, University of Helsinki, Unioninkatu 44, SF-00170 Helsinki 17, Finland

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Key Word Index—*Porphyridium purpureum*; Rhodophyceae; cell culture; glycolipids; fatty acids; detergents.

Abstract—The glycolipid fatty acid composition of *Porphyridium purpureum* on a solid medium was studied in the presence of Triton X-100 (TX), sodium desocycholate (SDC), sodium dodecyl sulphate (SDS) and cetyl trimethylammonium bromide (CTAB). TLC and GC/MS were used in determining the relative fatty acid compositions of mono- and digalactosyl diglycerides (MGDG and DGDG) and in assessing the MGDG/DGDG ratio. The most common fatty acids were palmitic (16:0), stearic (18:0), linoleic (18:2 ω6), arachidonic (20:4 ω6) and eicosapentaenoic (20:5 ω3) acids, the long-chain polyunsaturated acids being more abundant in DGDG; α-linolenic acid (18:3 ω3) was absent. TX and SDC in particular caused an increase in the saturation grade of both MGDG and DGDG fatty acids at very low concentrations (5–15 ppm). With a detergent concentration of 20 ppm a reversion of this tendency was sometimes found, and the fatty acid composition approached the controls again. The effects of SDS and CTAB were not as prominent. All the detergents studied increased the normal MGDG/DGDG ratio (0.3) to a maximum of $\sim$ 1. The effect of increasing detergent concentration is not linear. The results suggest that in some cases very low detergent concentrations can be more effective than higher ones, a fact which may be important in algae growing in polluted waters.

INTRODUCTION

Detergents, extensively used as washing agents, contribute to water pollution from industrial, household and oil spill fighting sources. They have also been used as valuable solubilizers in biochemistry. However, very little work has been done on their effects on living cells. It is well known that detergents usually inhibit algal growth [1], the cationic types being especially poisonous [2, 3], but occasionally even some growth-enhancing effects have been observed with non-ionics [1].

It can be assumed that the harmful effects of detergents on cells are linked to the detergent's ability to bind to membrane lipids and proteins and to alter their characteristics [4], e.g. permeability [5] already at very low concentrations. This depends, however, on the organism studied and its internal physiological state [6]. Hundreds of detergents exist today, and their behaviour and harmfulness towards cells varies widely. In this work we have used four detergents of different chemical types that have been commonly used in research [1, 4, 7]: similar types are common in commercial products and the bile salts have an important physiological role in the gut.

Not much is known about the reactions of halophytic algae towards detergents, although such information is very much in demand because of marine pollution. We used *Porphyridium purpureum* as a suitable object of study, one of the very few easily cultured red algae; ecologically it is very aero- and halophilous and tolerant towards external stresses [8, 9]. Its ultrastructure is also well known [10]. Something is known of its lipid composition, but in general its lipids have not been well studied [11, 12]. Its cultural demands and respiratory chain have been studied by Tulloss [13].

External stress and many chemical factors are known to affect the fatty acid composition of plant lipids [14]. It is well known that detergents usually inhibit photosynthesis [15, 16]. The aim of this study was to find out if detergents have an influence on the fatty acid composition of the chloroplast membranes, where MGDG* and DGDG are located in plants, and if they could alter the MGDG/DGDG ratio, which is also a function of growth conditions [14]. In preliminary experiments it was found that *P. purpureum* did not tolerate high concentrations of detergents in the medium: SDS and CTAB killed the cells if concentrations over 5 ppm (SDS 17 μM, CTAB 14 μM) were used and TX gradually decreased the growth by about 64% in a concentration area of 5–20 ppm (8–32 μM). SDC did not have any significant effect on growth in this area (5–20 ppm or 3–13 μM).

The detergent monomer form is the effective form in these concentrations, which are well below the critical micellar concentrations (CMC) of the detergents used [4]; although monomers and micelles coexist and the CMC is strongly decreased by ions [7], in the main the monomers are bound to membrane lipids and proteins. When the CMC is exceeded, most detergent molecules are arranged in micelles and this means a profound change in detergent action [4, 7]; in much higher concentrations the micelles start to solubilize membranes.

*Abbreviations used throughout are: MGDG, monogalactosyldiglycerides; DGDG, digalactosyldiglycerides; TX, Triton X-100; SDC, sodium desoxycholate; SDS, sodium dodecyl sulphate; CTAB, cetyl trimethylammonium bromide.

RESULTS

Normal fatty acid composition

The glycolipid fatty acid composition of *P. purpureum* is quite different from most other plants, reflecting the isolated status of the Rhodophyceae. A typical feature is the absence of α-linolenic acid (18:3 ω3). In its place the glycolipids, especially DGDG, contain large amounts of arachidonic acid (20:4 ω6) and eicosapentaenoic acid (20:5 ω3) (Tables 1–6). The most common fatty acid in both glycolipids was palmitic acid (16:0). Stearic (18:0) and linoleic (18:2 ω6) acids were also found to be fairly abundant, but the amount of oleic acid (18:1) was smaller. The C_{20} series was well represented, especially in DGDG, except for 20:0.

Some less abundant saturated acids could be found (14:0, 15:0, 17:0, 24:0) and certain, more unusual, unsaturated acids were also present, such as *trans* 16:1 ω3, 14:3 (together with 16:1) 22:1 and 24:1, although not in large amounts (see Tables). In some samples an incompletely identified, long-chain, unsaturated acid was present. Its ECL values corresponded to those of 22:5 ω3.

MGDG and DGDG are quite different as regards the occurrence of long-chain unsaturated acids. The relative amount of these acids is much larger in DGDG, while MGDG contains a larger amount of shorter chain acids, mainly those belonging to the C_{14}–C_{16} series. 16:1 was found in MGDG; MS data revealed that the GC 16:1 peak also contained 14:3.

Effect of detergents on the fatty acid composition of MGDG and DGDG

The effects of TX are presented in Tables 1 and 2. An increase could be seen in the relative amounts of 16:0 and other saturated acids as well as 20:2 ω9 in MGDG. A corresponding decrease occurred in the amounts of 18:2 ω6, 20:4 ω6 and 20:5 ω3 in both glycolipids as well as in the amount of 18:1 in MGDG. In the concentration of 20 ppm TX a reversion occurred in the case of 16:0. The saturation grade in both glycolipids was increased by TX.

An even clearer change was observed in the glycolipid fatty acid composition in the presence of SDC (Tables 3 and 4). Here too, the changes were more marked in DGDG and they followed roughly the outlines observed with TX: the amounts of 16:0, 18:0 and in MGDG also those of 14:0 and 17:0 increased between the concentrations of 5–15 ppm SDC, with a simultaneous decrease in the amounts of 20:2 ω9 in DGDG, of 18:1 in MGDG and of 18:2 ω6, 20:4 ω6 and 20:5 ω3 in both. The saturation grade increased markedly in this concentration range. Only a few differences from the controls were found in 20 ppm SDC, except in the amounts of 20:4 ω6 and 20:5ω3, the levels of which were a little higher in MGDG than in the controls. This phenomenon suggests a change in the behaviour of SDC between the concentrations of 15 and 20 ppm.

SDS and CTAB differed from the other two detergents both in regard to growth (see Introduction) and effects on the glycolipid fatty acid composition (Tables 5 and 6). The

Table 1. Percentage amounts of fatty acids in *P. purpureum*

MGDG in the presence of Triton X-100† (TX)					
		Conc. of Triton X-100 in growth medium (ppm)			
Acid	Control	5	10	15	20
14:0	1.4	3.4***	3.3***	tr	1.9
14:1	tr	tr	tr	tr	tr
14:3 + 16:1	2.4	tr	2.8	4.0	2.1
15:0	1.0	1.9	2.4	1.1	1.4
16:0	37.4	36.8	46.0*	44.7**	37.0
trans-16:1 ω3	1.7	2.9	2.3	1.2	0.9
16:3 ω3	—	—	—	—	—
16:3 ω6	—	0.9	—	—	—
17:0	tr	0.7	0.9	0.7	0.7
18:0	7.3	6.7	9.0	6.6	12.9
18:1	5.4	8.3	3.2*	3.3**	2.9**
18:2 ω6	14.8	6.1***	2.6***	11.7*	6.0***
18:3 ω6	tr	—	—	—	tr
20:1	tr	—	—	—	tr
20:2 ω9	0.5	8.8***	4.4**	tr	10.9**
20:3 ω6	0.8	—	tr	—	tr
20:4 ω6	10.4	5.1***	—	6.8**	2.2***
20:5 ω3	7.9	7.3	tr	9.0	3.0**
22:1	tr	—	tr	tr	tr
22:5 ω3	2.0	tr	5.6	1.3	tr
24:0	tr	—	tr	2.2	tr
24:1	2.8	1.5	6.5*	1.2	2.0

†Average percentages based on 8–10 independent samples, expressed as % of total fatty acid. All results were statistically tested with Student's *t* test: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, tr = < 0.5%.

Table 2. Percentual amounts of fatty acids in *P. purpureum* DGDG in the presence of Triton X-100 (TX)†

		Conc. of TX in growth medium (ppm)			
Acid	Control	5	10	15	20
14:0	tr	tr	1.2	0.8	0.8
14:1	—	—	tr	tr	tr
14:3+16:1	—	1.5	tr	1.3	1.3
15:0	tr	tr	0.9	0.6	0.8
16:0	49.2	54.0	60.8*	64.6**	47.1
trans-16:1 ω3	0.9	—	0.6	1.4	0.9
16:3 ω3	—	—	tr	—	—
16:3 ω6	—	—	—	—	—
17:0	tr	tr	tr	tr	tr
18:0	3.3	5.1**	10.6***	6.6*	9.8**
18:1	2.1	1.9	3.4	1.7	3.6
18:2 ω6	6.2	3.7***	3.5**	3.7***	4.6***
18:3 ω6	—	—	—	—	—
20:1	tr	tr	—	—	tr
20:2 ω9	2.2	2.7	2.8	2.2	3.3*
20:3 ω6	0.8	0.5	tr	—	0.6
20:4 ω6	11.2	6.9**	1.4***	2.7***	3.9***
20:5 ω3	20.2	19.4	6.7***	9.4***	11.2***
22:1	—	—	—	tr	—
22:5 ω3	0.8	1.7	tr	—	3.8
24:0	tr	—	tr	tr	1.0
24:1	tr	tr	1.2	tr	0.8

†See Table 1 for explanation of symbols used.

Table 3. Percentual amounts of fatty acids in *P. purpureum*†

MGDG in the presence of sodium desoxycholate (SDC)					
		Conc. of SDC in growth medium (ppm)			
Acid	Control	5	10	15	20
14:0	1.4	tr	3.6***	3.9***	0.8
14:1	tr	tr	—	1.5	tr
14:3+16:1	2.4	0.5	3.3	2.3	4.3
15:0	1.0	tr	2.8***	1.9***	0.6
16:0	37.4	27.3**	52.9**	43.6	31.6
trans-16:1 ω3	1.7	1.6	3.0	0.8	1.1
16:3 ω3	—	—	—	—	—
16:3 ω6	—	—	—	0.8	tr
17:0	tr	0.7	1.0	1.5	0.6
18:0	7.3	12.2	11.1*	14.1***	7.6
18:1	5.4	11.8*	6.8	2.1***	4.3
18:2 ω6	14.8	7.6**	6.2***	1.6***	15.5
18:3 ω6	tr	tr	—	tr	tr
20:1	tr	tr	—	tr	—
20:2 ω9	0.5	tr	2.9	1.6	tr
20:3 ω6	0.8	tr	—	tr	1.0
20:4 ω6	10.4	7.4	tr	1.5***	17.3**
20:5 ω3	7.9	16.1***	tr	2.4***	10.7*
22:1	tr	—	tr	2.4	—
22:5 ω3	2.0	—	—	tr	0.7
24:0	tr	—	—	2.8	0.8
24:1	2.8	1.5	2.6	5.0	0.5

†For explanation of symbols used, see Table 1.

Table 4. Percentual amounts of fatty acids in *P. purpureum* DGDG in the presence of sodium desoxycholate (SDC)†

		Conc. of SDC in growth medium (ppm)			
Acid	Control	5	10	15	20
14:0	tr	tr	tr	0.7	tr
14:1	—	—	—	tr	tr
14:3 + 16:1	—	tr	—	—	tr
15:0	tr	tr	0.7	0.7	0.5
16:0	49.2	47.0	59.6***	64.9***	46.6
trans-16:1 ω3	0.9	tr	1.3	tr	1.1
16:3 ω3	—	—	—	—	—
16:3 ω6	—	—	—	—	tr
17:0	tr	tr	tr	0.6	tr
18:0	3.3	5.7*	10.0***	19.3***	4.5
18:1	2.1	1.6	2.6	1.0	2.1
18:2 ω6	6.2	3.2***	4.1***	1.8***	6.4
18:3 ω6	—	—	—	tr	—
20:1	tr	tr	—	—	—
20:2 ω9	2.2	3.6	2.7	0.9*	1.5
20:3 ω6	0.8	tr	tr	—	0.8
20:4 ω6	11.2	6.5	2.9***	1.0***	11.6
20:5 ω3	20.2	25.0	12.8**	2.3***	22.6
22:1	—	tr	—	tr	—
22:5 ω3	0.8	3.7	—	tr	tr
24:0	tr	—	—	tr	tr
24:1	tr	tr	1.4	1.5	tr

†See Table 1 for explanation of symbols used.

Table 5. Percentual amounts of fatty acids in *P. purpureum* MGDG in the presence of sodium dodecyl sulphate (SDS) and cetyl trimethylammonium bromide (CTAB)†

		Conc. of SDS and CTAB in growth medium (ppm)			
		SDS		CTAB	
Acid	Control	2.5	5	2.5	5
14:0	1.4	2.6	1.8	4.0**	2.2
14:1	tr	1.1	—	1.3	tr
14:3 + 16:1	2.4	2.9	2.2	1.7	1.9
15:0	1.0	1.7	1.1	3.4	1.4
16:0	37.4	36.5	32.7	35.7	29.8*
trans-16:1 ω3	1.7	1.7	2.7	3.2	2.2
16:3 ω3	—	tr	tr	tr	—
16:3 ω6	—	tr	—	1.2	0.7
17:0	tr	1.0	0.5	0.8	0.6
18:0	7.3	10.4*	8.2	9.5	7.8
18:1	5.4	3.3*	6.3	5.2	6.6
18:2 ω6	14.8	6.9**	6.8***	2.1**	6.5***
18:3 ω6	tr	tr	—	tr	—
20:1	tr	—	—	—	—
20:2 ω9	0.5	1.9**	5.6***	1.4	7.6***
20:3 ω6	0.8	0.6	—	tr	—
20:4 ω6	10.4	4.2***	10.6	tr	6.3**
20:5 ω3	7.9	3.0**	8.1	1.2***	8.6
22:1	tr	3.6	tr	4.7	tr
22:5 ω3	2.0	—	6.5***	—	6.7**
24:0	tr	6.9**	—	3.8	tr
24:1	2.8	3.4	3.4	2.7	5.5

†See Table 1 for explanation of symbols used.

Table 6. Percentual amounts of fatty acids in *P. purpureum* DGDG in the presence of sodium dodecyl sulphate (SDS) and cetyl trimethylammonium bromide (CTAB)†

		Conc. of SDS and CTAB in growth medium (ppm)			
		SDS		CTAB	
Acid	Control	2.5	5	2.5	5
14:0	tr	0.7	2.3	0.8	tr
14:1	—	tr	tr	—	—
14:3+16:1	—	—	tr	tr	—
15:0	tr	0.7	1.4	tr	tr
16:0	49.2	57.0**	40.3*	53.5*	31.2*
trans-16:1	0.9	1.5	3.5	1.4	0.9
16:3 ω3	—	—	—	—	—
16:3 ω6	—	—	2.1	0.7	tr
17:0	tr	tr	tr	tr	tr
18:0	3.3	8.8***	6.1*	8.9***	6.6**
18:1	2.1	1.9	5.1	2.1	3.1
18:2 ω6	6.2	4.8**	3.4***	4.0	4.0**
18:3 ω6	—	—	tr	—	—
20:1	tr		tr	—	—
20:2 ω9	2.2	2.7	2.9	2.5	3.8
20:3 ω6	0.8	tr	tr	tr	—
20:4 ω6	11.2	2.9***	5.9*	2.4***	8.6
20:5 ω3	20.2	12.5**	14.0	12.5*	36.7
22:1	—	1.3	—	1.1	tr
22:5 ω3	0.8	—	1.9	—	2.0
24:0	tr	0.9	tr	0.8	—
24:1	tr	1.7	1.2	1.0	tr

†See Table 1 for explanation of symbols used.

changes were not as prominent as in the presence of TX and SDC and could be observed mainly in 18:2 ω6, 20:4 ω6 and 20:5 ω3, the relative amounts of which, particularly with SDS, seemed to decrease somewhat. However, the saturation grade did not increase very markedly.

The amount of 20:2 ω9 showed a substantial increase in MGDG with an increase in detergent concentration. The same observation was made in DGDG 20:5 ω3 in the presence of CTAB. It seems that these changes do not explain the general harmfulness of CTAB. Similarly no response had earlier been observed with CTAB as regards the phosphatases in *Nitzschia* [17].

MGDG/DGDG ratio

Normally the MGDG/DGDG ratio of *P. purpureum* when cultured in these conditions seems to be very low, on average 0.35 (s.e. 0.02, Table 7). This is in contrast with the results from most other plant groups [14, 18, 19]. Our results showed that all detergents tested increased the MGDG/DGDG ratio. This is especially marked in the presence of SDS and CTAB, where the value can be as high as > 1. TX and SDC at low concentrations also increased the ratio. Detergents proved to be similar to many other physical and chemical factors in being able to change the MGDG/DGDG ratio [14, 19].

Table 7. MGDG/DGDG ratio in *P. purpureum* cultured in the presence of Triton X-100 (TX), sodium desoxycholate (SDC), sodium dodecyl sulphate (SDS) and cetyl trimethylammonium bromide (CTAB)†

Detergent	Conc. (ppm)	MGDG/DGDG
—	0	0.35 (control)
TX	5	0.83*
	10	0.41
	15	0.55*
	20	0.54
SDC	5	0.27*
	10	0.84*
	15	0.56
	20	0.44
SDS	2.5	1.09**
	5	0.39
CTAB	2.5	0.95*
	5	1.24**

†Average of five independent determinations.

DISCUSSION

Nichols and Appleby [11] have already published some results on the fatty acid compositions of *P. purpureum* MGDG and DGDG, and in general our results are in line with their findings. Compared with higher plants, the

glycolipid fatty acid composition is simple and is characterized by a great difference between the amounts of 'major' and 'minor' acids. Some 'minor' acids of other plant groups, e.g. those belonging to the unsaturated C_{16} series [20], are almost entirely absent from *P. purpureum*.

The C_{18} and C_{20} series are abundant in *P. purpureum* glycolipids with the exception of 18:3 ω3 and 20:0. It is stated [11] that in *P. purpureum* 20:4 ω6 seems to be synthesized via a pathway typical of mammals, which involves 18:3 ω6 and 20:3 ω6 as intermediates. We could confirm the existence of these isomers; other types of 18:3, 20:3 or 20:4 were not found. The C_{20} polyenoic acids perhaps participate in chlorophyll stabilization, partly replacing 18:3 ω3 [21]. The known connection of 18:3 ω3 with O_2 evolution does not exist in *P. purpureum*, which is an obligate photoautotroph [13]. Furthermore it only contains small amounts of 18:3 ω6, not 18:3 ω3.

The relative amount of saturated acids was quite high in *P. purpureum* glycolipids, which may also partly be a result of the high growth temperature used (25°). This has been found to increase the algal lipid saturation grade [20, 22, 23]. According to our results detergents also seem to increase the degree of saturation. This tendency has also been observed with other harmful chemicals; correspondingly, membrane permeability is increased [5, 24].

The low MGDG/DGDG ratio observed in *P. purpureum* is also a function of the growth temperature, but an equally important reason may be the red algal chloroplast structure with few thylakoids [10]. Allen *et al.* [22] have studied the thermophilous alga *Cyanidium*, which is considered to be related to the Rhodophyceae, and found a similar ratio there. Corresponding results have also been obtained from plastids in non-photosynthetic tissues, which contain few thylakoids [25, 26]. The plastid envelope is richer in DGDG, although qualitatively thylakoids and envelopes contain both glycolipids [27].

In *P. purpureum* the MGDG/DGDG ratio always seems to rise in the presence of detergents in the growth medium. Perhaps the changes in chloroplast envelopes are greater than in the thylakoids, which is also indicated by the more marked fatty acid changes in DGDG. The ratio is always subject to the influence of external factors, but in different plant groups the changes do not have the same direction [14].

All detergents are able to bind to membrane proteins and lipids, often at specific binding sites, and this usually changes the membrane properties. Of the detergents used here, SDS and CTAB are noted for their denaturing effects [4]. CTAB is even commercially used in disinfection [3] because of its bactericidal activity. The action of TX and SDC is not so well understood as those of the denaturing detergents [4]. Our results indicate that TX and SDC also have an ability to cause changes in membranes at low concentrations in spite of their biologically 'mild' characteristics. To affect membranes the detergent monomer must traverse the plant cell wall as well as the extracellular slime in *P. purpureum* [9], which places different detergents in a different position according to their solubility properties: long hydrophobic chains like those in TX, SDS and CTAB probably slow down the penetration [4]. Detergent-induced changes in the lipid fatty acid composition may also be results of indirect influence through fatty acid biosynthesis. In polluted natural waters the effects of detergents on living organisms are bound to be complex. In some cases, very low detergent concentrations may be more effective than higher ones: our results with TX and SDC showed the greatest differences from controls at 5–10 ppm concentrations. Perhaps the reversions to the original fatty acid compositions observed at 15–20 ppm concentrations of TX and SDC were due to a change in the monomer/micelle ratio of the detergent. Earlier results on phosphatases in *Nitzschia* [17] show a fairly similar pattern as regards the behaviour of the detergents. This could mean that even a slight amount of detergent pollution in natural waters is a matter which cannot be ignored.

EXPERIMENTAL

Plant material. The unicellular, halophilous red alga *Porphyridium purpureum* (Bory) Ross (*syn. P. cruentum* (Ag. Naeg.) strain CCAP 1380/1a (Cambridge, England) was used as a pure culture.

Culture. The alga was cultured on a solid 1% agar nutrient medium after [13] containing the following macro nutrients (ppm): NaCl 27000, $MgSO_4$ 3200, $MgCl_2$ 2600, $CaCl_2$ 1300, KNO_3 1000, KH_2PO_4 70, and $NaHCO_3$ 40. The medium was buffered with 1 M Tris–HCl pH 7.6 (20 ml/l), Fe was added as an 8.3 mg/ml Fe^{3+} Na-EDTA soln (1 ml/l). The micronutrient soln used (1 ml/l) contained (in ppm): $ZnCl_2$ 40, H_3BO_3 600, $CoCl_2$ 8.2, $CuCl_2$ 31.6, $MnCl_2$ 254, and $(NH_4)_6MoO_{24}$ 370.

Detergents. Four different types of detergents were used: (1) Triton X-100 (TX, iso-octylphenoxypolyethoxyethanol, nonionic, CMC 0.24 mM in H_2O), (2) sodium desoxycholate (SDC, anionic, CMC 4–6 in H_2O), (3) sodium dodecyl sulphate (SDS, anionic, CMC 0.52 in 0.5 M NaCl), (4) acetyl trimethylammonium bromide (CTAB, cationic, CMC about zero in ionic solutions); their characteristics are well known [2, 4, 7, 28]. All the detergents used were of analytical grade and were added to the slightly cooled autoclaved medium using 0.22 μm sterile Millipore filters to avoid their possible decomposition during sterilization.

Growth and harvest. Growth time was one month in Ø 7 cm Petri dishes at 25° under Airam 40W-35 white fluorescent tubes giving on average 100 $\mu E/m^2/sec$ (400–700 nm) 18 hr/day. After growth time the tough algal layer was removed quantitatively from the agar surface and lyophilized. Every sample consisted of the combined harvested algae of four Petri dishes.

Lipid analysis. The lipids were extracted with $CHCl_3$–MeOH (2:1) and fractioned on a silicic acid column [29]. The glycolipids were separated using TLC plates of silica gel 60 F_{254}, two spots from every sample, from which one was used for MGDG/DGDG ratio determinations and the other for GC. The plates were developed with $CHCl_3$–MeOH–7 M NH_3 (65:20:4) [30] and the glycolipids located with very slight amounts of I_2 vapour blown only onto the ratio spots [31]. Extraction from the plates was done using $CHCl_3$–MeOH (2:1). The MGDG/DGDG ratios were determined spectrophotometrically [32].

The MGDG and DGDG fatty acid compositions were determined by GC/MS of the methyl esters [33]. The methyl esters were analysed using a Varian 3700 GC combined with a Vista 401 CDS. A WCOT glass capillary column Silar 10-C of 30 m (ID 0.3 mm) with an injection vol. of 2 μl was used, temp. programmed 120° to 185° at 3°/min with a total run time of 1 hr. Injector and detector heater temps were 210°. N_2 at 30 ml/min was used as carrier gas. For MS the samples were run with a Fractovap 2150 GC using a SP-1000 WCOT glass capillary column of 50 m (i.d. 0.33 mm) with an initial temp. of 60°. After the solvent retention time an abrupt swift was made to 150°, then the temp. was programmed to 210° at 4°/min. The GC was combined with a JEOL JMS-1000 MS and a JEOL Mass Data System. The interface temp. was 260°, the ionization current

300 μA and voltage 30 eV. FID was used in all GC.

ECL values were used according to the principle of Jamieson [34] to determine the double bond positions in the polyunsaturated fatty acid methyl esters.

Statistics. The results are average percentages based on 8–10 independent samples, expressed as % of total fatty acid. The statistical significance of all results was tested with Student's *t* test: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

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