Pergamon

PII: S0031-9422(97)00201-X

Phytochemistry, Vol. 45, No. 8, pp. 1561–1567, 1997

0031-9422/97 $17.00+0.00

LIPIDS OF *PAVLOVA LUTHERI*: CELLULAR SITE AND METABOLIC ROLE OF DGCC

WALDEMAR EICHENBERGER* and CORINNE GRIBI

Department of Chemistry and Biochemistry, University of Bern, Freiestrasse 3, 3012 Bern, Switzerland

(*Received in revised form* 25 *January* 1997)

Key Word Index *Pavlova lutheri*; Haptophyceae; betaine lipids; DGCC; DGGA; DGTA; fatty acids; MGDG.

Abstract—*Pavlova lutheri* contains triacylglycerols (TAG), monogalactosyldiacylglycerols (MGDG), galactosyldiacylglycerols (DGDG), sulfoquivonosyldiacylglycerols (SQDG), diacylglyceryl hydroxymethyl-*N*,*N*,*N*-trimethyl-β-alanine (DGTA), diacylglyceryl carboxyhydroxymethylcholine (DGCC) and diacylglyceryl glucuronide (DGGA) as major lipid components. Prominent fatty acids are 14:0, 16:0, 16:1, 18:4, 20:5, 22:5 and 22:6. In MGDG and DGDG, 18:4 and 20:5 predominate, while SQDG contains high proportions of 14:0 and 16:0. DGCC is enriched in 16:0 and 20:5, DGGA in 18:1, 22:5 and 22:6, and DGTA in 22:6. Analysis of subcellular membrane fractions demonstrated an accumulation of DGCC, DGTA and DGGA in non-plastid membranes. On incubation of cells with [1-^{14}C] oleic acid, after 60 min of pulse, 30% of the incorporated label was found in TAG and 70% in polar lipids. DGCC (52%) and DGTA (12%) were the most strongly labelled polar lipids. Within 72 h of chase, in DGCC and in TAG the label rapidly decreased to 11% and 17%, respectively, but in MGDG it increased in the same time up to 35% of the total. Only minor changes were observed in DGTA and no significant label was recorded in DAG during the chase-period. The shift of label from 18:1 to 18:4, 20:5 and 22:6 reflected further elongation and/or desaturation of the substrate. Very similar results obtained using [2-^{14}C] acetate as precursor suggested that DGCC acts as a primary acceptor of *de novo*-formed or exogenous fatty acids which subsequently undergo processing and redistribution. It is suggested that C_{18} and C_{20} fatty acids are transferred individually from the cytoplasm to the chloroplast allowing the synthesis of eukaryotic MGDG without the import of DAG.

INTRODUCTION

Marine algae are exceptional with regard to their lipid pattern. Many of them produce large amounts of long-chain C_{20} and C_{22} polyenoic fatty acids [1–5]. Most of these organisms also contain glycerolipids of the betaine-type in the form of either diacylglyceryl-*N*,*N*,*N*-trimethylhomoserine (DGTS), diacylglyceryl-hydroxymethyl-*N*,*N*,*N*-trimethyl-β-alanine (DGTA) or diacylglycerylcarboxyhydroxymethylcholine (DGCC) [6–10]. Of these lipids, a zwitterionic structure is typical resembling that of phosphatidylcholine (PC). Surprisingly, many algae containing betaine lipids do not contain detectable amounts of PC like certain green algae [10, 11], Phaeophyceae [12] and Haptophyceae [10, 13, 14]. Finally, a typical feature of algae (except cyanobacteria and most green algae) is the structure of the plastid lipid monogalactosyldiacylglycerol (MGDG), which in these organisms is mainly occupied in its *sn*-2 position by C_{18} or longer acyl chains [2, 15, 16]. In higher plants, MGDG of this 'eukaryotic'-type is synthesized from diacylglycerol (DAG) originating from cytoplasmic PC, which is also involved in lipid-linked fatty acid desaturation [17, 38]. Thus, the formation of eukaryotic MGDG is especially intriguing in algae, which do not contain detectable amounts of PC, suggesting that lipid constituents other than PC are involved in MGDG biosynthesis. The idea that algae may synthesize eukaryotic MGDG in a way which is different from that of higher plants, has been put forward already by Sato [18]. Little is also known about the biosynthesis of long-chain polyunsaturated fatty acids in plastid lipids. The synthesis of arachidonic acid (20:4) in *Euglena gracilis* [19] and of 20:5 in *Nannochloropsis* [20] has been ascribed to the cytoplasm. The use of C_{20} acyl groups for MGDG synthesis in the chloroplast would involve an exchange of fatty acids between different cellular compartments. Candidates for a function in acyl-exchange reactions are the betaine lipids, since DGTS and DGTA act as intermediate acyl

* Author to whom correspondence should be addressed.

Table 1. Fatty acid and lipid composition of *Pavlova lutheri*

Fatty acid (mol%)	Total lipid	TAG	MGDG	DGDG	SQDG	DGGA	DGTA	DGCC	PG	U1	U2
14:0	7.4	8.6	3.1	4.6	34.7	1.1	t	2.9	0.7	7.7	4.1
16:0	38.4	39.2	8.3	19.0	44.8	4.9	5.8	35.5	9.1	15.3	17.1
16:1	24.8	32.9	8.7	9.6	9.7	t	1.6	2.1	3.2	40.6	39.5
16:1 (3t)	—	—	—	0.9	—	—	—	—	12.8	—	—
16:3	t	—	—	—	t	—	—	—	2.2	—	—
18:0	0.5	t	—	—	t	3.3	t	1.4	1.1	—	1.8
18:1 (n-9)+(n-11)	2.2	2.1	—	—	5.8	19.2	0.5	1.7	3.7	2.2	3.4
18:2 (n-6)	1.5	3.7	2.5	t	t	1.9	t	1.0	3.7	20.2	4.1
18:3 (n-6)	0.7	—	4.0	2.0	t	—	—	—	t	t	t
18:3 (n-3)	0.8	—	3.5	1.5	t	t	—	—	0.5	0.5	2.4
18:4 (n-3)	6.1	1.1	26.1	13.3	0.6	0.8	1.1	t	1.6	2.4	5.8
20:2	t	4.3	t	t	1.3	1.3	0.5	t	t	0.6	2.2
20:4 (n-6)	0.5	—	t	t	t	0.5	—	4.6	0.5	—	0.9
20:5 (n-3)	13.8	7.0	43.8	48.6	2.6	3.7	5.4	39.6	34.6	6.0	8.3
22:5 (n-6)	0.5	—	—	—	0.5	44.4	5.4	0.6	2.5	—	—
22:6 (n-3)	3.3	1.1	—	0.7	t	18.9	73.7	10.6	24.3	4.5	10.4
Others	t	—	—	—	—	—	6.0	—	1.0	—	—
Lipid mol%	100	42.0	18.9	11.8	9.3	1.9	5.8	4.9	0.9	1.8	2.8

Values are means of two determinations $t = < 0.5\%$.

carriers in *Ochromonas* [21] and DGTA in *Chroomonas* [22, 23]. A similar role has recently been attributed to DGCC in *Pavlova lutheri* [10]. This alga was used in our experiments, too, because it produces C_{20} and C_{22} fatty acids and contains DGTA and DGCC but not PC [6, 24]. Our aim was to gain insights into the incorporation and processing of [1-^{14}C] oleic acid, which acts as precursor of C_{20} and C_{22} fatty acids [25], and into the utilization of these fatty acids for MGDG synthesis. Also, the cellular site of the two betaine lipids was examined.

RESULTS AND DISCUSSION

The lipid and fatty acid composition of *Pavlova* are presented in Table 1. TAG, MGDG, DGDG, SQDG, DGTA and DGCC were found as major lipid components. In addition, diacylglycerylglucuronide (DGGA) could also be identified indicating that this anionic lipid also occurs in algae other than *Ochromonas*, where it has recently been detected [26]. Phosphatidylglycerol (PG) was present in minor amounts only, while phosphatidylethanolamine (PE), PC and DGTS could not be detected in *Pavlova*. Two unknown fatty-acid containing compounds U_1 and U_2 were considered as diacyl lipids, but their structure could not be identi-fied. TAG accounted for 42 mol% and polar lipids for 58 mol% of the total lipids.

In total, fatty acids, 16:0, 16:1 and 20:5 predominate comprising 77% of the total. The high proportion of 16:0 and 16:1 acids is due to their predominance in TAG, where C_{16} fatty acids account for 72% of the total. MGDG and DGDG contain mainly 18:4 and 20:5, with an enhanced amount of 16:0 in DGDG, while SQDG is enriched in 14:0 and 16:0 acids. DGCC contains mainly 16:0 and 20:5. In contrast, DGTA is enriched in 22:6 and DGGA contains mostly 18:1, 22:5 and 22:6.

The positional distribution of fatty acids in MGDG, DGCC and DGTA are presented in Table 2. In MGDG, the *sn*-2 position is occupied almost 80% by C_{18}, C_{20} or C_{22} acyl chains, indicating that this lipid is predominantly of the eukaryotic type with 20:5 mainly in the *sn*-1 and C_{18} acids in the *sn*-2 position. Thus, 20:5–18:3 and 20:5–18:4 combinations are suggested to dominate in MGDG. In DGCC, in contrast, 16:0–20:5, 16:0–22:6, 20:5–20:5 and 20:5–22:6 combinations, and in DGTA 22:6–22:6, 20:4–

Table 2. Positional distribution of fatty acids in MGDG, DGCC and DGTA of *Pavlova lutheri*

Fatty acid (mol%)	MGDG		DGCC		DGTA	
	sn-1	*sn*-2	*sn*-1	*sn*-2	*sn*-1	*sn*-2
14:0	t	4.0	t	t	—	—
16:0	0.6	12.6	39.4	2.0	6.2	t
16:1	6.6	4.8	3.8	1.0	6.1	—
18:0	t	1.6	2.6	0.8	0.9	—
18:1	t	1.8	2.0	1.1	—	—
18:2	t	5.9	t	0.9	—	—
18:3	t	16.3	—	1.8	—	—
18:4	t	47.5	t	4.0	—	—
20:4	1.1	—	t	2.5	13.7	0.8
20:5	91.4	1.3	41.2	52.0	8.3	t
22:5	—		0.8	t	7.0	1.9
22:6	t	4.2	9.6	33.7	57.8	96.6
C_{16}	7.2	17.4	43.2	3.0	12.3	t
$C_{18}+C_{20}+C_{22}$	92.5	78.6	56.2	96.8	87.7	99.3

Values are means of two determinations $t = < 0.5\%$.

22:6 and 16:0–22:6 species have to be expected. Both DGCC and DGTA are almost exclusively of the eukaryotic structure containing high amounts of 20:5 and 22:6 in their *sn*-2 positions.

In order to investigate the incorporation of oleic acid into the different lipids and its further processing and distribution, cells of *Pavlova* were incubated with [1-^{14}C] oleate in a pulse-chase manner. Lipids were extracted at the end of the pulse and at different times of the chase-period and separated by 2D-TLC. Spots were scraped off and eluted, and an aliquot was used for measuring the radioactivity in particular lipids. From another aliquot, fatty acids were converted to phenacyl esters and separated by RP-HPLC and the label monitored.

The incorporation of radioactivity into different lipids is illustrated in Fig. 1. At the end of the 1-h pulse, 30% of the incorporated label was found in TAG and 70% in the polar lipids. Of these, the most strongly labelled components were DGCC (52%) and DGTA (12%); DGGA, SQDG and MGDG contained only minor portions of label. During the subsequent 72 h of chase, the label first increased up to 46% and then decreased to 17% of the total in TAG. In DGCC, the radioactivity rapidly decreased to 11%, but in the same time drastically increased in MGDG up to 35% of the total. A delayed increase was also observed in DGDG and SQDG, while only minor changes occurred in DGGA. In DGTA, a decrease of label followed by an increase was observed. Almost no radioactivity was found in PG, PE, diacylglycerol (DAG) and in the unidentified components U_1 and U_2 (results not shown). This indicates that the fatty acid substrate taken up by the cell was channelled primarily into the betaine lipids, DGCC and DGTA, and into TAG. These results suggest that DGCC and possibly also TAG, act as primary acceptors for fatty acids to be subsequently used for the biosynthesis of MGDG. DGTA, in contrast, although an acceptor of fatty acids, does not appear to undergo a similar turnover.

When the total label in polar lipids, including glycolipids and betaine lipids, was considered, an increase was observed during the first 30 min of the pulse, but thereafter the total label remained almost constant (Fig. 2). This may indicate that after the end of the pulse, a redistribution of fatty acids took place primarily between the polar lipids, although an exchange between TAG and polar lipids could not be excluded. It is noteworthy that similar labelling kinetics in DGCC, DGTA and MGDG was observed, if [2-^{14}C] acetate, [1-^{14}C] laurate, [1-^{14}C] myristate or [1-^{14}C] palmitate were applied as substrates (results not shown). This indicates that the same flux of fatty acids passing through DGCC holds not only for exogenous but also for *de novo*-synthesized fatty acids. A rapid labelling of DGCC in *Pavlova* was also observed by Kato *et al.* [14]. But simultaneous strong labelling of MGDG measured by these authors is in contradiction with

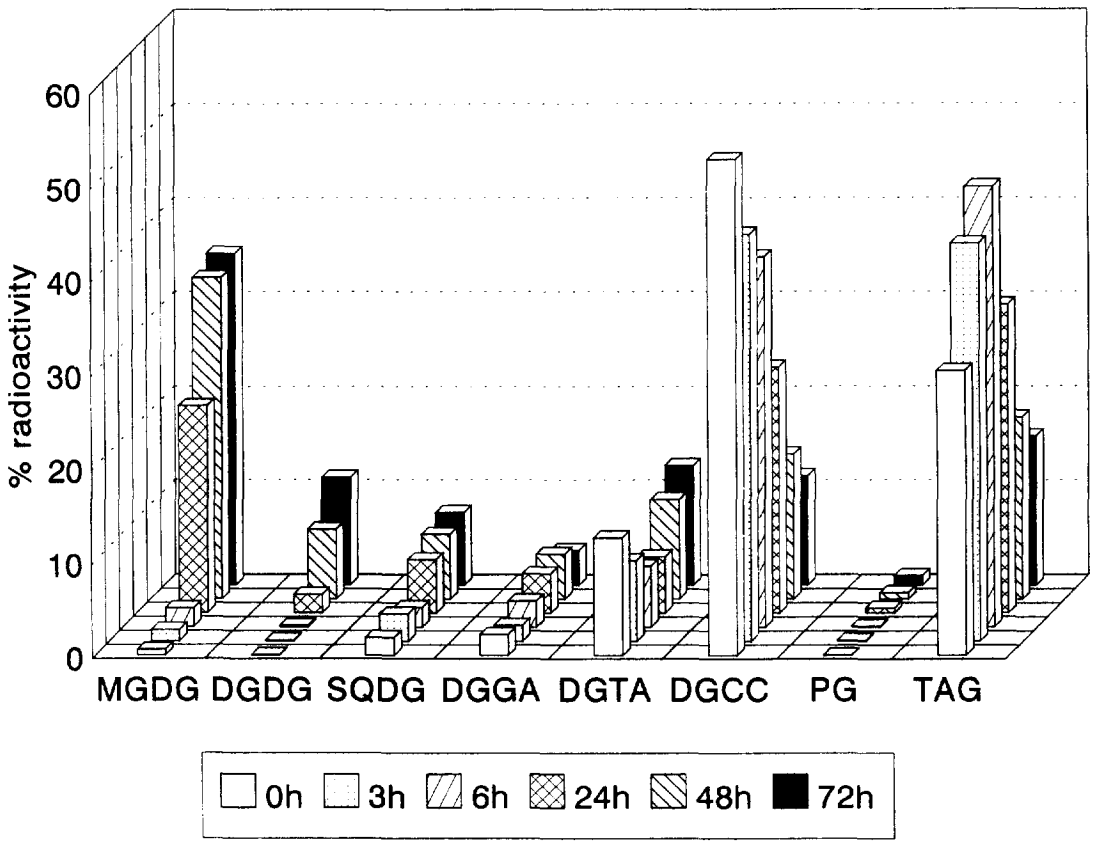

Fig. 1. Incorporation of [1-^{14}C] oleate into lipids of *Pavlova lutheri* after a 0, 3, 6, 24, 48 and 72 hr chase.

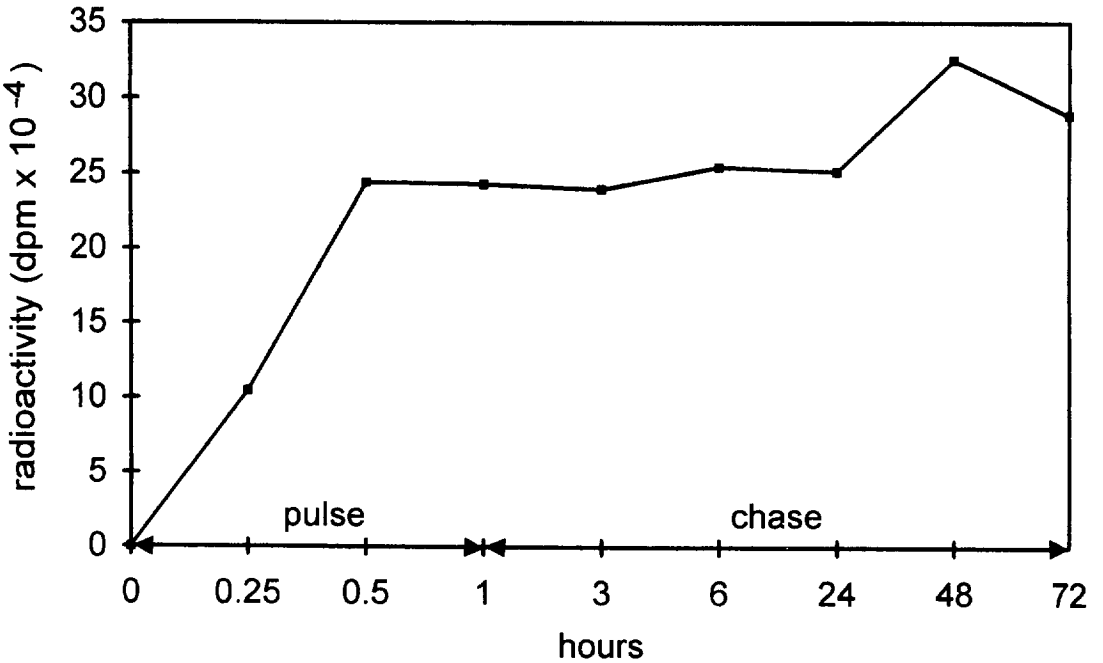

Fig. 2. Incorporation of [1-^{14}C] oleate into lipids of *Pavlova lutheri*. Total radioactivity in polar lipids.

our findings and may be explained by the different experimental conditions used.

Since the results obtained strongly suggest a prominent role of DGCC in the incorporation and redistribution of fatty acids within the cell, the question arose about the cellular localization of this lipid. Thus, cells of *Pavlova* were homogenized in a French pressure cell and the homogenate fractionated by differential and density-gradient centrifugation. Two particle fractions enriched in chloroplast and non-chloroplast membranes, respectively, were isolated and analysed for both chlorophyll and polar lipids. The results, summarized in Table 3, indicate that relative to chlorophyll, the contents of both MGDG and DGDG are very similar in the homogenate, in the enriched chloroplast fraction and, due to plastid impurities, also in the enriched non-chloroplast fraction, as expected for chloroplast constituents. The content of DGCC and DGTA, in contrast, is low in the chloroplast fraction but high in non-chloroplast membranes. From these values, it is calculated that for total cellular DGCC and DGTA, a maximum of 12 and 11%, respectively, can be attributed to the chloroplast membranes. This indicates that neither DGCC, DGTA nor DGGA are localized in chloroplasts, but are constituents of extraplastid membranes. It should be noted that the values for pure chloroplast membranes are even lower, since part of the DGTA and DGCC found in the chloroplast preparation has to be ascribed to non-plastid impurities. The lower content of MGDG in both subcellular fractions compared with intact cells can be explained by a specific degradation of MGDG during cell fractionation, since a similar low value for MGDG is also observed in the cell homogenate. Whether the accumulation of SQDG in the non-chloroplast fraction may be interpreted in terms of an extraplastid pool of this glycolipid, should be verified in further experiments.

Since, during the labelling experiments, desaturation and/or elongation of oleic acid had to be expected, the distribution of label among different fatty acids was examined. Of the three most strongly labelled lipids, i.e. MGDG, DGCC and DGTA, at different times, fatty acid phenacyl esters were prepared, separated by HPLC and monitored. The results obtained are shown in Fig. 3.

At the end of the pulse, within DGCC, the label

Table 3. Lipid composition of cells and enriched subcellular fractions from *Pavlova lutheri*

	nmol Lipid 100 μg $^{-1}$ chlorophyll a						
	MGDG	DGDG	SQDG	DGGA	DGCC	DGTA	PG
Intact cells	269	210	116	17	111	61	8
Cell homogenate	127	208	97	14	91	50	11
Chloroplast fraction	132	202	43	0	14	7	7
Non-chloroplast fraction	144	164	273	39	158	127	15

Values are means of two determinations.

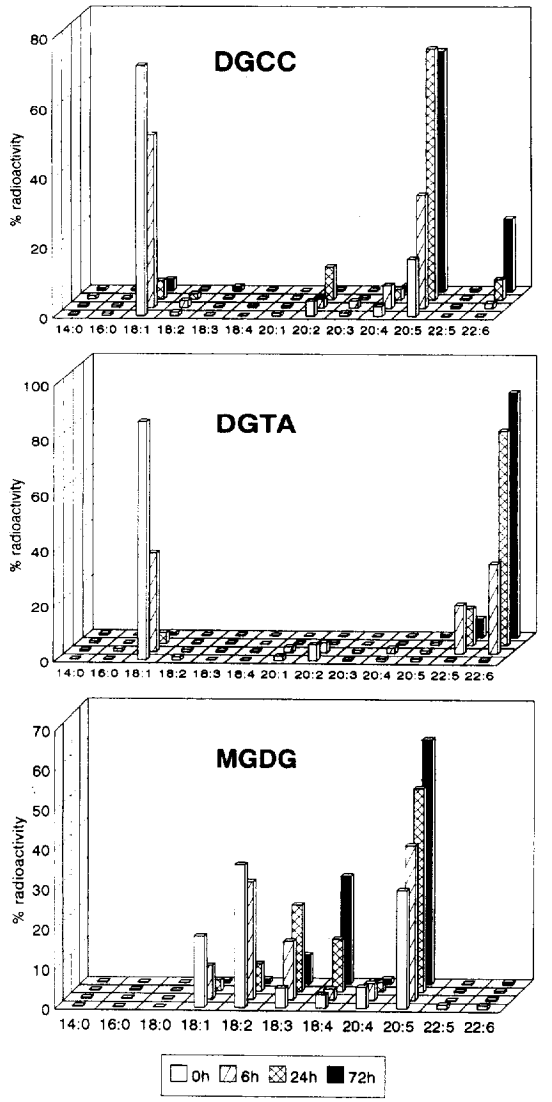

Fig. 3. Incorporation of [1-^{14}C] oleate into fatty acids of DGCC, DGTA and MGDG of *Pavlova lutheri* after 0, 6, 24 and 72 hr chase.

was mainly found in 18:1, with minor amounts in 20:5. During the chase, the radioactivity rapidly disappeared from 18:1 with a concomitant increase in 20:5 and also 22:6. In DGTA, at the beginning, most of the label was found in 18:1, then shifted to 22:6 and, intermediately, also to 22:5. This is in accordance with the time-course of labelling in this lipid (Fig. 1) suggesting that DGTA is acting as a final acceptor of C_{22} fatty acids. In MGDG, already at the end of the pulse, most of the label appeared in 18:2, 18:1 and 20:5 with minor amounts in 18:3, 18:4 and 20:4. Subsequently, the label drastically decreased in 18:2, 18:1 and 20:4, but accumulated in 18:4 and 20:5. 18:3, in contrast, was only intermediately labelled suggesting that this fatty acid underwent a turnover. This time-course of labelling strongly suggests a desaturation sequence from 18:1 or 18:2 through 18:3 to 18:4 acid occurring on MGDG itself. The same reaction sequence has also been observed in MGDG of the closely related alga, *Isochrysis galbana* [27]. The accumulation of 18:4 in *Pavlova* is in accordance with the view that for chain elongation a 18:3 rather than an 18:4 acid is used as an intermediate, as shown in *Porphyridium cruentum* [28] and *Nannochloropsis* [29]. Interestingly, 20:5 appears in MGDG almost simultaneously with 18:1 and 18:2, suggesting that, as in DGCC, 20:5 is introduced as such into this lipid. It is worth mentioning that during the experiment with [1-^{14}C] oleate, no radioactivity could be detected in fatty acids with chain lengths shorter than C_{18}, indicating that breakdown of 18:1 and re-incorporation of label by *de novo* biosynthesis did not occur. The formation of C_{20} and C_{22} from C_{18} acyl chains implicates one or two elongation steps, which in higher plants are catalysed by elongases of the endoplasmic reticulum using CoA esters as substrates [25]. The site and the substrates for desaturations are not clear, although many plant desaturases are known to act on lipid-bound fatty acids [17, 30].

Based on our results, it is concluded that extraplastid DGCC is involved in the transfer of fatty acids from the cytoplasm to the chloroplast and, thus, in the biosynthesis of MGDG. The incorporation of exogenous oleic acid into MGDG is suggested to occur in two ways. On the one hand, 18:1 or 18:2 are transferred from DGCC to MGDG where they remain esterified to the lipid and are further desaturated to 18:3 and 18:4, but not used for elongation. On the other hand, C_{18} fatty acids are inserted into MGDG only after elongation and desaturation, as 20:4 and/or 20:5 acids. Interestingly, the proportion of radiolabelled C_{20} fatty acids increases with time suggesting that C_{18} and C_{20} acyl groups are introduced independently into MGDG. The cytoplasmic origin of C_{20} fatty acids in MGDG is in keeping with the view that the elongation process very likely occurs in the cytoplasm [20, 25]. An import of 20:5 into the chloroplast from an extraplastid site has also been postulated for *Nannochloropsis* [29]. This translocation of fatty acids from one compartment to the other raises the question about the nature of the vehicle used. In plants, DAG originating from cytoplasmic PC is thought to be imported and used for the synthesis of eukaryotic MGDG in chloroplasts [31]. In algae, however, eukaryotic chloroplast lipids may be synthesized in a different way, as proposed by Sato [18]. Our data from *Pavlova* suggest that individual fatty acids rather than DAGs are transferred from the cytoplasm to the chloroplast and are incorporated into MGDG by an exchange mechanism. Future investigations should therefore focus on the biosynthesis of MGDG in algal chloroplasts. Also, the role of TAGs in algal lipid metabolism will have to be studied.

EXPERIMENTAL

Pavlova lutheri strain 931/1 was obtained from C.C.A.P., Dunstaffnage Laboratory, Oban, U.K.

Cells were cultivated in conical flasks (150 ml) in 30 ml of autoclaved medium prepd from natural seawater (North Sea, salinity 28‰) by enrichment with PES supplement as specified in ref. [32]. Cultures were shaken at 20° under 1300 lux permanent fluorescent light.

Cell rupture and fractionation. The method of ref. [33] was used. A suspension containing 1.02×10^{10} cells was centrifuged at 1700 *g* (2700 rpm) for 15 min. The pellet obtained was suspended in 3 ml medium containing 0.6 M sorbitol, 50 mM tricine-HCl and 20 mM KCl (pH 7.4) and the suspension passed once through a French pressure cell at 69 MPa. The homogenate was diluted with H_2O and centrifuged at 3000 *g* for 20 min. The pellet containing whole cells, debris, chloroplasts and chloroplast fragments, was suspended in H_2O and loaded onto a step gradient containing layers of 27, 34, 42 and 50 wt% sucrose. The gradients were centrifuged at 113 000 *g* for 1 hr. Chloroplasts banded as a dark brown band in the region of 42% sucrose and were recovered after dilution by centrifugation at 27 000 *g*. The fr. enriched in non-chloroplast membranes was obtained by centrifugation of the first supernatant (3000 *g*) at 100 000 *g* for 1 hr. For lipid analysis of the homogenate, an aliquot was kept at 4° during the cell fractionation procedure. Chlorophyll a was determined in 80% Me_2CO according to ref. [34].

Lipid isolation and analysis. Cells were collected by centrifugation and extracted with the 10-fold vol. of MeOH. Total lipids were sepd by 2D-TLC on silica gel (Merck 5715) using $CHCl_3$–MeOH–H_2O (65:25:4) (solvent 1) and $CHCl_3$–MeOH–*iso*PrNH_2–conc. NH_3 (130:70:1:10) (solvent 2). Spots were detected with 2′,7′-dichlorofluorescein under UV 366 nm and eluted with MeOH. DGCC was identified by its position in the chromatogram and by its Dragendorff-positive but molybdenum blue negative reaction indicating the presence of the trimethylammonium group and the absence of phosphorus. DGGA was identified by its chromatographic properties, by the specific colour reaction obtained with anthrone reagent and by the MS of the TMSi derivative of the deacylated lipid showing diagnostic fragments at *m/z* 685 $[M-Me]^+$ and 595 $[M-Me-TMSOH]^+$ of a hexa(trimethylsilyl)glycerylhexuronide [26]. DAG and TAG were isolated from total lipid by TLC on silica gel plates (Merck 5715) using $CHCl_3$–MeOH (50:1). The positional distribution of fatty acids among the *sn*-1 and *sn*-2 position was determined using lipase from *Rhizopus arrhizus* [35]. Fatty acids Me esters were prepared either from pure lipids by transesterification with NaOMe [36] or from free fatty acids by treatment with CH_2N_2 according to ref. [37]. Quantitative determination of lipids was done by FID-GLC of the constituent fatty acids using 20:0 Me ester as int. standard. A fused silica capillary column (30 m length, 0.25 mm I.D.) coated with Carbowax 20 M and H_2 as carrier gas were used. The temp. was 185–210° at 2° min^{-1}, 210° for 1 min and then 210–220° at 5° min^{-1}. Fatty acid phenacyl esters were prepd from phenacyl bromide according to ref. [38] and sepd on a Nucleosil 100-5 C_{18} (250 × 4 mm) column using MeCN (A) and MeCN–H_2O (3:2) (B), as solvents. The gradient was 20 to 80% A in 80 min and for detection, UV 242 nm was used.

Incubation conditions. Cells harvested by centrifugation (*ca* 2.2×10^9) were suspended in 3 ml of culture medium and added to a soln of 4 μCi (148 MBq) [1-^{14}C] oleic acid (57 mCi $mmol^{-1}$, Amersham) in 20 μl EtOH. Incubation was done on a shaker under illumination at 20°. After a 1-hr pulse, cells were centrifuged, washed with 6 ml chase medium containing 70 μg unlabelled oleic acid, resuspended and then distributed among five conical flasks containing 40 ml chase medium and kept under culture conditions. Aliquots were taken after 0, 3, 6, 24, 48 and 72 hr of chase.

Radioactivity was measured by liquid scintillation counting after adding 2 ml MeOH and 5 ml butyl-PBD (Ciba-Geigy) 7% (w/v) in toluene.

EI MS was carried out using an SE-30 capillary column.

Acknowledgements—This work has been supported by the Swiss National Science Foundation (Grant 31-45901.95).

REFERENCES

1. Harwood, J. L. and Jones, A. L., *Advances in Botanical Research*, 1989, **16**, 1.
2. Arao, T. and Yamada, M., *Phytochemistry*, 1989, **28**, 805.
3. Fleurence, J., Gutbier, G., Mabeau, S. and Leray, C., *Journal of Applied Phycology*, 1994, **6**, 527.
4. Zhukova, N. V. and Aizdaicher, N. A., *Phytochemistry*, 1995, **39**, 351.
5. Vaskovsky, V. E., Khotimchenko, S. V., Xia, B. and Hefang, L., *Phytochemistry*, 1996, **42**, 1347.
6. Kato, M., Adachi, K., Hajiro-Nakanishi, K., Ishigaki, E., Sano, H. and Miyachi, S., *Phytochemistry*, 1994, **37**, 279.
7. Sato, N., *Botany Magazine Tokyo*, 1992, **105**, 185.
8. Eichenberger, W., *Plant Physiology and Biochemistry*, 1993, **31**, 213.
9. Dembitsky, V. M., *Progress in Lipid Research*, 1996, **35**, 1.
10. Kato, M., Sakai, M., Adachi, K., Ikemoto, K. and Sano, H., *Phytochemistry*, 1996, **42**, 1341.
11. Giroud, C., Gerber, A. and Eichenberger, W., *Plant Cell Physiology*, 1988, **29**, 587.
12. Eichenberger, W., Araki, S. and Müller, D. G., *Phytochemistry*, 1993, **34**, 1323.
13. Kato, M., Adachi, K., Ikemoto, H. and Sano, H., *Plant Cell Physiology*, 1995, **36** (Suppl.), 31.
14. Kato, M., Hajiro-Nakanishi, K., Sano, H. and Miyachi, S., *Plant Cell Physiology*, 1995, **36**, 1607.
15. Araki, S., Sakurai, T., Kawaguchi, A. and Murata, N., *Plant Cell Physiology*, 1987, **28**, 761.

16. Jones, A. L. and Harwood, J. L., *Phytochemistry*, 1992, **31**, 3397.
17. Ohlrogge, J. and Browse, J., *The Plant Cell*, 1995, **7**, 957.
18. Sato, N., *Plant Cell Physiology*, 1991, **32**, 845.
19. Nichols, B. W. and Appleby, R. S., *Phytochemistry*, 1969, **8**, 1907.
20. Schneider, J. C. and Roessler, P., *Journal of Phycology*, 1994, **30**, 594.
21. Vogel, G. and Eichenberger, W., *Plant Cell Physiology*, 1992, **33**, 427.
22. Henderson, R. J. and Mackinlay, E. E., *Plant Cell Physiology*, 1992, **33**, 395.
23. Eichenberger, W., Gfeller, H., Gray, P., Gribi, C. and Henderson, R. J., *Phytochemistry*, 1996, **42**, 967.
24. Tatsuzawa, H. and Takizawa, E., *Phytochemistry*, 1995, **40**, 397.
25. Cassagne, C., Lessire, R., Bessoule, J. J., Moreau, P., Creach, A., Schneider, F. and Sturbois, B., *Progress in Lipid Research*, 1994, **33**, 55.
26. Eichenberger, W. and Gribi, C., *Journal of Plant Physiology*, 1994, **144**, 272.
27. Stern, N. and Tietz, A., *Biochimica et Biophysica Acta*, 1993, **1167**, 248.
28. Khozin, I., Adlerstein, D., Bigogno, C. and Cohen, Z., in *12th International Symposium on Plant Lipids, Physiology, Biochemistry and Molecular Biology of Plant Lipids*, ed. J. P. Williams, M. U. Khan and N. W. Lem. Kluwer Academic, Dordrecht, 1997, p. 93. Toronto, Canada, 1996, (in press).
29. Schneider, J. C., Livne, A., Sukenik, A. and Roessler, P. G., *Phytochemistry*, 1995, **40**, 807.
30. Harwood, J. L., *Annual Review of Plant Physiology and Plant Molecular Biology*, 1988, **39**, 101.
31. Roughan, P. G. and Slack, C. R., *Annual Review of Plant Physiology*, 1982, **33**, 97.
32. Starr, R. C. and Zeikus, J. A., *Journal of Phycology*, 1987, **23** (Suppl.), 1.
33. Hiller, R. G., Bardin, A.-M. and Nabedryk, E., *Biochimica et Biophysica Acta*, 1987, **894**, 367.
34. Lichtenthaler, H. K., Bach, T. J. and Wellburn, A. R., in *Biochemistry and Metabolism of Plant Lipids*, ed J. F. G. M. Wintermans and P. J. C. Kuiper. Elsevier, Amsterdam, 1982, p. 489.
35. Fischer, W. and Heinz, E., *Hoppe Seyler's Zeitschrift für Physiologische Chemie*, 1973, **354**, 1115.
36. Thies, W., *Zietschrift für Pflanzenzuchtung*, 1971, **65**, 83.
37. Eichenberger, W., *Phytochemistry*, 1976, **15**, 459.
38. Borch, R. F., *Analytical Chemistry*, 1975, **47**, 2437.