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Lipids of *Chlamydomonas reinhardtii*. Analysis of Molecular Species and Intracellular Site(s) of Biosynthesis

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Membrane lipids of *Chlamydomonas reinhardtii* were separated into the major components, MGDG, DGDG, SQDG, DGTS, PG, PE, PI, and the molecular species of each lipid were isolated and analyzed. The fatty acid composition was determined for the total lipid, the particular lipid classes and the molecular species. The positional distribution of fatty acids between the C-1 and C-2 position of the glycerol moiety was also determined. MGDG, DGDG, SQDG, PG and probably PI were found to be of plastidic (prokaryotic) origin, while DGTS and PE were found to be of cytoplasmic (eukaryotic) origin. Prokaryotic lipids mainly contained 18:3(9,12,15), 18:2,16:4 and 16:3, while DGTS and PE were rich in 18:3(5,9,12), 18:4(5,9,12,15), 18:2 and 18:1(11) fatty acids. From the fact that each lipid class was characterized by an individual pattern of molecular species, we conclude that during their biosynthesis, all the lipids act individually as substrates for the lipid-linked desaturation of fatty acids. Moreover, our results suggest that in *Chlamydomonas*, 18:3(5,9,12) and 18:4(5,9,12,15) are formed in the cytoplasm using DGTS and PE as substrates.

Key words: Biosynthesis — *Chlamydomonas* — Desaturation — Fatty acids — Lipid — Molecular species.

The membrane lipids of *Chlamydomonas reinhardtii* have been repeatedly studied with respect to their composition and biosynthesis (Eichenberger 1976, Janero and Barrnett 1981 a, b, c, 1982a, b). The lipid pattern of this alga is rendered unique by the presence of diacylglyceryltrimethylhomoserine (DGTS) (Eichenberger and Boschetti 1978, Janero and Barrnett 1982c), a betaine lipid which is also produced by most other cryptogamic plants (Sato and Furuya 1985) and of which the biosynthetic pathway is not yet known. Furthermore, it is a matter of controversy, whether or not the widespread phospholipid phosphatidylcholine (PC) is present in *Chlamydomonas*. Janero and Barrnett (1981b) reported considerable amounts of this lipid, while others (Eichenberger 1982, Sato and Furuya 1985) could not detect it in this alga.

Only a few studies have dealt with the intracellular site(s) of lipid biosynthesis in *Chlamydomonas*. Jelsema et al. (1982) and Michaels et al. (1983) have shown that two important enzymes of the glycerolipid synthesis, namely glycerol phosphate acyltransferase and lysophosphatidate acyltransferase, are detectable in envelopes, thylakoids and pyrenoids of chloroplasts as well as in the Golgi vesicles. This indicates that diglycerides may be formed in different cellular compartments. For green plants, it is generally accepted that the diglyceride moieties of glycerolipids are synthesized either by the chloroplast (prokaryotic pathway) or by the endoplasmic reticulum (eukaryotic pathway) (Roughan and Slack 1982, 1984), as summarized by Frentzen (1986) and by Heemskerk and Wintermans (1987).

Abbreviations: BHT, butyl hydroxytoluene; DGDG, digalactosyldiacylglycerol; DEGS, diethyleneglycolsuccinate; DGTS, diacylglyceryl(*N,N,N*-trimethyl)homoserine; FID, flame ionization detector; GLC, gas liquid chromatography; HPLC, high performance liquid chromatography; I.D., inner diameter; MGDG, monogalactosyldiacylglycerol; MS, mass spectrometry; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PG, phosphatidylglycerol; PI, phosphatidylinositol; SQDG, sulfoquinovosyldiacylglycerol; TLC, thin-layer chromatography; 16:0, hexadecanoic acid; 16:1, hexadecenoic acid; 16:1(3t), 3-*trans* hexadecenoic acid; 16:2, hexadecadienoic acid; 16:3, hexadecatrienoic acid; 16:4, hexadecatetraenoic acid; 18:0, octadecanoic acid; 18:1(9), 9-octadecenoic acid; 18:1(11), 11-octa decenoic acid; 18:2(9,12), octadecadienoic (linoleic) acid; 18:3 (9,12,15) or α18:3, 9,12,15-octadecatrienoic (α-linolenic) acid; 18:3(6,9,12) or γ18:3, 6,9,12-octadecatrienoic (γ-linolenic) acid; 18:3(5,9,12) or i18:3, 5,9,12-octadecatrienoic acid; 18:4(5,9,12,15), 5,9,12,15-octadecatetraenoic acid.

Lipids originating from either pathway are recognized by a characteristic positional distribution of fatty acids at the glycerol backbone. In the eukaryotic pathway, PC plays a central role as the substrate of fatty acid desaturase(s) (Demandre et al. 1986, Murphy et al. 1985). This raises the interesting question as to what extent the prokaryotic and eukaryotic pathways contribute to the synthesis of major membrane lipids of *Chlamydomonas* which does not dispose of PC as a substrate for fatty acid desaturase(s).

The aim of the present work was to analyze the positional distribution of fatty acids in different lipids and to identify their molecular species by HPLC and GLC methods. The results should allow us to identify the cellular site(s) of lipid synthesis and the possible biosynthetic pathways leading to the different fatty acids and lipids.

Materials and Methods

Plant materials—Chlamydomonas reinhardtii 137c arg-2$^-$ mt$^+$ (The Culture Centre of Algae and Protozoa, Cambridge) was cultivated autotrophically under 10,000 lux of continuous fluorescent light for 7 days at 26–28°C. The nutrient was medium I (Sager and Granick 1954) with 0.47 mM arginine-HCL. *C. reinhardtii* cell wall-less mutant CC-406 (*Chlamydomonas* Genetics Center, Duke University, Durham, NC) was cultivated under the same light conditions in high salt medium (Sueoka 1960) supplemented with yeast extract (0.4% w/v) and Na-acetate (0.12% w/v).

Ochromonas danica (The Culture Centre of Algae and Protozoa, Cambridge) was cultivated on a modified medium (Pringsheim 1955) containing (in wt%) 0.2% trypton (Difco), 0.1% yeast extract (Difco), 0.1% Liebig's meat extract and 0.5% glucose. Cells were cultivated under illumination for 5 days at 26–28°C.

Lipid isolation—Lipids were extracted with methanol containing 0.05% butyl hydroxytoluene (BHT) as an antioxidant. They were separated on precoated silica gel plates (Merck 5715) with chloroform/methanol/acetic acid/water (85 : 15 : 10 : 3, by vol.) (Nichols et al. 1965) and rechromatographed with different solvents: chloroform/methanol (5 : 1, v/v) for MGDG, chloroform/methanol/water (70 : 30 : 4, by vol.) for DGDG, DGTS, PE, SQDG and PG, and chloroform/methanol/acetic acid/water (30 : 15 : 4 : 2, by vol.) for PI. Spots were detected under UV (366 nm) after spraying with 2′,7′-dichlorofluoresceine and eluted with methanol. After evaporation of the solvent, the lipid was purified by partition between 1%(w/v) Na_2CO_3 and chloroform.

Fatty acid analysis—Fatty acid methyl esters were obtained either from total lipids by saponification with methanolic KOH and esterification with diazomethane (Eichenberger 1976) or from pure lipids by transesterification with sodium methoxide (Thies 1971). For analytical GLC separations, a Shimadzu GC-8A equipped with FID was used. The column was a fused silica capillary column, 25 m long, 0.25 mm I.D., coated with Carbowax 20 M (chemically bound) operating at 190–210°C (1°C·min^{-1}) with H_2 as carrier gas. Eicosanoic (20:0) acid methyl ester was used as an internal standard. Integration of peaks was done with a Shimadzu Chromatopac C-R3A. For preparative purposes, a Perkin-Elmer 990 instrument equipped with a stream splitter and FID was used. C_{16} and C_{18} fatty acid methyl esters were separated from each other on a packed steel column (5% DC-200 silicon oil on Chromosorb W, 1 m length, 6 mm I.D., 200°C, 86 ml N_2·min^{-1}). For the isolation of single C_{16} or C_{18} fatty acid methyl esters, 5% DEGS on Chromosorb W (4 m long, 6 mm I.D., 185°C, 40 ml N_2·min^{-1}) was used.

The positional distribution of the fatty acids among the C-1 and C-2 position of the glycerol backbone was determined using the lipase from *Rhizopus arrhizus* (Fischer et al. 1973).

Picolinyl esters of fatty acids were prepared according to Christie et al. (1986). Mass spectra were obtained with a GC-MS Varian MAT 44S instrument equipped with a Spectro System MAT 200, at 70 eV. Oxidation of fatty acids with $KMnO_4$ was carried out according to Kates (1986). The resultant mono- and dicarboxylic acid fragments were separated as methyl esters on Carbowax 20 M (fused silica, 25 m length, 0.3 mm I.D., 140–200°C (2°C·min^{-1}) with H_2 as carrier gas, and identified by comparison with reference mono- and dicarboxylic acid methyl esters.

Molecular species analysis—Lipids were separated into their constitutent molecular species by reversed phase HPLC on a Perkin-Elmer Series 10 Liquid Chromatograph. A 20-μl solution of lipid in methanol was loaded on a column (250×4 mm) containing Nucleosil 5 C 18 (5 μm particle size, Knauer, FRG). Solvents and flow rates used for the isocratic separation of different lipid classes are as follows: Methanol/water (94 : 6, v/v), flow 1 ml/min for MGDG; methanol/water (90 : 10, v/v), 1 ml/min for DGDG (adapted from Yamauchi et al. 1982); methanol/water/acetonitrile (90.5 : 7 : 2.5, by vol.) containing 20 mM choline-HCl, 1 ml/min for both SQDG and PE, and 1.5 ml/min for PI (adapted for PE and PI from Patton et al. 1982, Robins and Patton 1986); acetonitrile/methanol/acetic acid/1-ethylpropylamine (611 : 56 : 1.7 : 1, by vol.), 1.5 ml/min for PG (adapted from Smith et al. 1985); acetonitrile/methanol/acetic acid/1-ethylpropylamine (550 : 116 : 1.7 : 1, by vol.), 1 ml/min for DGTS (adapted from Norman and Thompson 1985). The effluent was monitored at 202 nm with a Perkin-Elmer LC-75 detector connected to a LCI-100 integrator. For the identification and quantitative determination of molecular species, fractions corresponding to single peaks were collected, taken to dryness under N_2 in the presence of BHT and then con-

verted to methyl esters by transesterification. The amount of each molecular species was determined from the quantities of its constituent fatty acids.

Radiolabelling with [^{32}P]phosphate—Cells of *Chlamydomonas* (strain 137c and CC-406) and of *Ochromonas* were grown for 5 days in 35 ml of nutrient containing 0.5 mCi (18.5 MBq) [^{32}P]orthophosphate (Amersham). Lipids were extracted and chromatographed on silica gel (Merck 5715) with chloroform/methanol/water (65 : 25 : 4, by vol.) in the 1st dimension and chloroform/methanol/isopropylamine/conc. NH_3 (65 : 35 : 0.5 : 5, by vol.) in the 2nd dimension. For autoradiography, Agfa-Gevaert Curix X-ray film was exposed for 2 hours for *Ochromonas* and 8 hours for *Chlamydomonas*.

Results

The membrane lipids of *Chlamydomonas* consist of MGDG, DGDG, SQDG, DGTS, PG, PE and PI as major components, as described earlier (Eichenberger 1976). In order to reinvestigate the presence or absence of PC, two strains of *Chlamydomonas* (137c and cell wall-less mutant CC-406) and *Ochromonas* (as a reference organism) were incubated with [^{32}P]orthophosphate. The lipids were then separated by TLC and autoradiographed. Under the conditions used, PE, PG, PC and PI were well separated, as shown in Fig. 1. While PC was clearly present in *Ochromonas* (Fig. 1a), the spot was lacking in the two species of *Chlamydomonas* (Fig. 1b, c). This indicates that this lipid is radiochemically not detectable in *Chlamydomonas*.

The total fatty acid composition is given in Table 1. The dominant chain lengths were C_{16} and C_{18}, accounting for 46 and 54%, respectively. Longer-chain fatty acids were present in trace amounts only. Main components were 16:0, 16:1(7), 16:1(9), 16:1(3t), 16:2(7,10), two isomers of 16:3, 16:4, 18:0, 18:1(9), 18:2(9,12), 18:3(9,12,15), 18:3(5,9,12) and 18:4(5,9,12,15). The tentative structure of the two C_{16}-trienes is $\Delta^{4,7,10}$ and $\Delta^{7,10,13}$, and that of the tetraene $\Delta^{4,7,10,13}$. Within the C_{18} series, apart from 18:1(9), 18:1(11) acid could also be detected in considerable amounts. 18:3(5,9,12) was previously considered to be γ-linolenic acid [18:3(6,9,12)] (Eichenberger 1976).

Evidence for the structure of C_{18} fatty acids comes from the MS and GLC data shown in Table 2. The 18:1(11) methyl ester was characterized by its molecular weight and by both the C_7 monocarboxylic and the C_{11} dicarboxylic acid fragments obtained by oxidation. The structure of 18:3(5,9,12) could be ascertained by the molecular ion and typical fragments of the picolinyl ester which were all identical to those obtained with 18:3(6,9,12) indicating double bonds in the 9- and 12-positions (Christie et al. 1986). The Δ^5-double bond was confirmed by the C_5 dicarboxylic acid fragment obtained by oxidation. From 18:3(6,9,12), which could not be detected in *Chlamydomonas*, a C_6 fragment, and from 18:3(9,12,15) a C_9 fragment was produced under the same conditions. The

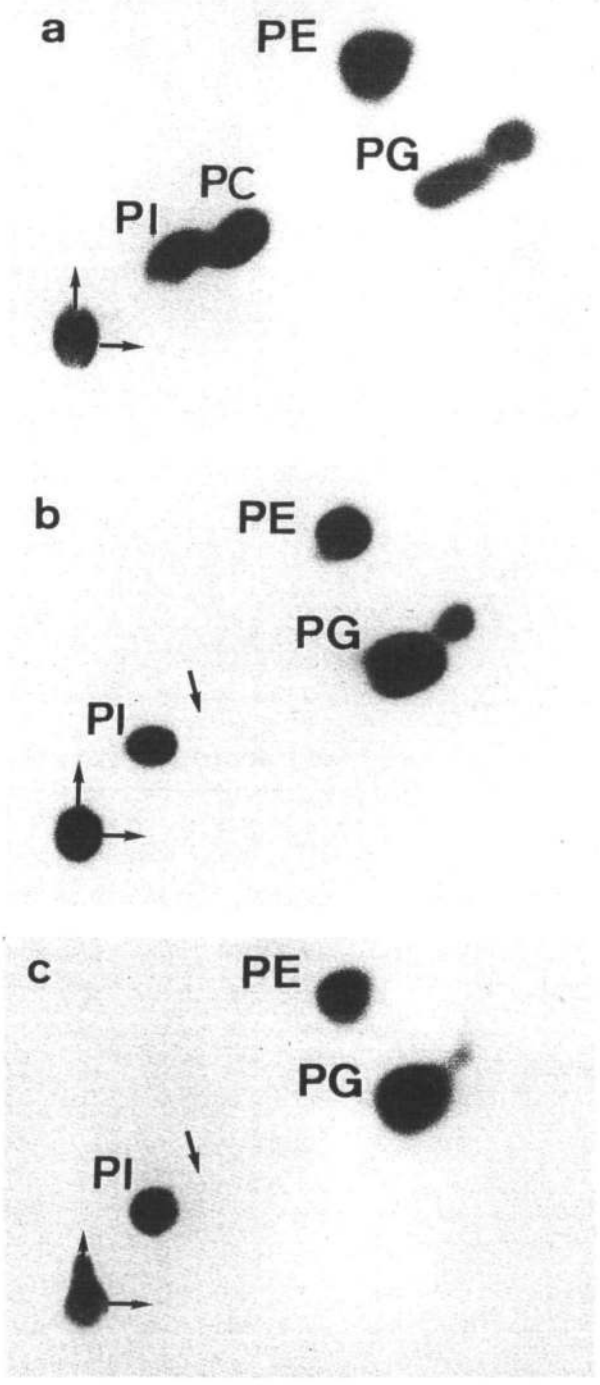

Fig. 1 Autoradiogram of ^{32}P-labelled and TLC-separated phospholipids from a) *Ochromonas danica*, b) *Chlamydomonas reinhardtii* strain 137c, c) *Chlamydomonas reinhardtii* cell wall-less mutant CC-406. Conditions were as indicated in Materials and Methods.

Table 1 Fatty acid composition of total lipids from *Chlamydomonas reinhardtii* 137c

Mol% fatty acids														
16:0	16:1 (7)	16:1 (9)	16:1 (3t)	16:2 (7,10)	16:3 (4,7,10)	16:3 (7,10,13)	16:4 (4,7,10,13)	18:0	18:1 (9)	18:1 (11)	18:2 (9,12)	18:3 (5,9,12)	18:3 (9,12,15)	18:4 (5,9,12,15)
22	5	tr	2	2	1	2	13	2	13	3	8	9	16	2

tr, Trace.

Table 2 MS and GLC data of the C_{18} fatty acids of *Chlamydomonas reinhardtii* 137c

Fatty acid	Retention time of methyl ester (Carbowax 20 M)	Fragments obtained by oxidation of methyl esters	MS signals (m/e) of picolinyl esters						
			M^+	M-29	M-55	M-71 M-69	M-97 M-95	M-111 M-109	M-137 M-135
18:0	1.00								
18:1(9)	1.05	C_{9m}, C_{9d}	373						
18:1(11)	1.07	C_{7m}, C_{11d}	373						
18:2(9,12)	1.17	C_{9d}	371						
18:3(5,9,12)	1.23	C_{5d}	369			298	272	258	232
18:3(6,9,12)	1.26	C_{6d}	369			298	272	258	232
18:3(9,12,15)	1.36	C_{9d}	369	340	314	300	274	260	234
18:4(5,9,12,15)	1.44	C_{5d}	367	338	312	298	272	258	232

m, Monocarboxylic acid. d, Dicarboxylic acid.

Table 3 Composition and positional distribution of fatty acids in glycerolipids of *Chlamydomonas reinhardtii* 137c

Lipid	Position	Mol% fatty acids												C_{16}	C_{18}
		16:0	16:1[b]	16:2	16:3[b]	16:4	18:0	18:1 (9)	18:1 (11)	18:2 (9,12)	18:3 (5,9,12)	18:3 (9,12,15)	18:4 (5,9,12,15)		
MGDG	1	2	1	tr	tr	tr	1	27	1	15	1	52	tr	3	97
	2	2	14	7	10	64	tr	tr	—	tr	1	tr	tr	97	3
DGDG	1	4	tr	tr	—	tr	2	55	2	16	1	19	tr	4	96
	2	76	14	4	2	2	1	tr	—	tr	1	tr	—	97	3
SQDG	1	68	—	—	—	—	2	6	9	5	1	10	—	68	32
	2	99	tr	—	tr	—	—	—	—	tr	tr	1	—	99	1
PG	1	6	2[a]	—	—	—	1	11	11	47	—	21	—	8	92
	2	27	51[a]	—	11	—	7	tr	—	1	1	tr	1	88	12
PI	1	21	1	—	1	—	4	7	52	5	3	5	—	24	76
	2	83	2	2	—	—	2	1	11	—	1	—	—	86	14
DGTS	1	58	2	tr	tr	tr	2	2	5	17	5	7	1	61	39
	2	3	7	1	1	2	—	1	1	11	59	3	9	14	86
PE	1	8	tr	—	tr	—	49	1	33	4	3	1	tr	8	92
	2	7	1	—	3	—	tr	—	tr	tr	79	3	6	11	89

[a] in the case of PG: 16:1 (3t)

[b] All isomers.

tr, Trace

—, Not detected.

$\Delta^{5,9,12,15}$ structure of the 18:4 was decided from the MS fragments which are typical for double bonds in the 9-, 12- and 15-positions (Harvey 1984). The corresponding fragments were also obtained with 18:3(9,12,15). The Δ^5-double bond was also confirmed by the C_5 dicarboxylic acid fragment obtained by oxidation. Moreover, the retention times of 18:3(5,9,12) and 18:4(5,9,12,15) were identical with those of authentic fatty acids isolated from Norway spruce wood (not shown in the table) (Ekman 1980).

The different fatty acids were characteristically distributed among the various lipids, as shown in Table 3. 16:4 and 18:3(9,12,15) were concentrated in MGDG. DGDG was enriched in 18:3(9,12,15), 18:1(9), 18:2 and 16:0. For SQDG, a large amount of 16:0, and for PG, a large proportion of 16:1(3t), 16:0 and 18:2 were typical. 18:3(5,9,12) and 18:4(5,9,12,15) were concentrated in DGTS and PE. 18:1(11) was mainly found in DGTS, PE, PG, SQDG and PI. DGTS and PI contained 16:0, and PE 18:0 as a saturated component. It is interesting to note that both 18:3(9,12,15) and 16:4 were almost exclusively localized in MGDG and DGDG, while both 18:3(5,9,12) and 18:4 were almost limited to DGTS and PE.

In each lipid, the positional distribution of fatty acids was analyzed by splitting off the acyl group in the C-1 position with the lipase from *Rhizopus arrhizus*. It turned out to be a common feature of MGDG, DGDG, SQDG, PG and also PI that in the C-2 position of their glycerol moiety, C_{16} acids accounted for >85 mol% of the total fatty acids linked to this position, indicating that these lipids were predominantly prokaryotic. In contrast, DGTS and PE contained >85 mol% C_{18} acids in the same position and were therefore of eukaryotic origin. It is interesting to note that in these two lipids, both 18:3(5,9,12) and 18:4 were almost strictly limited to the C-2 position. In contrast, 18:1(9) and 18:1(11) were concentrated in the C-1 position.

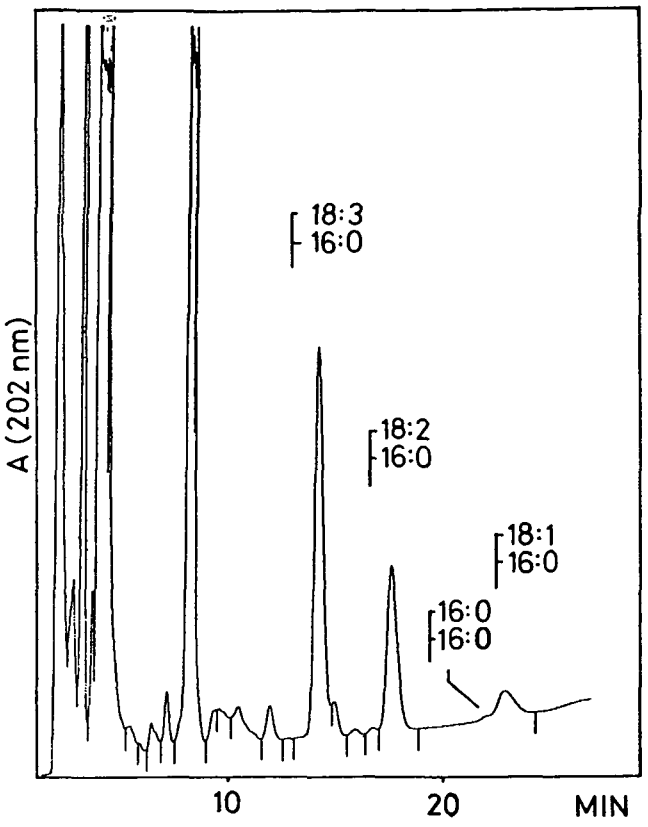

Fig. 2 Separation by HPLC of molecular species of underivatized sulfoquivonosyldiacylglycerol (SQDG). Conditions were as described in Materials and Methods.

Table 4 Molecular species of glycerolipids of *Chlamydomonas reinhardtii* 137c

		Fatty acid combinations (C1/C2) in mol% of total															
MGDG	%	DGDG		%	DGTS	%	SQDG	%	PG	%	PI	%	PE	%
a18:3/16:4	70	a18:3/	16:4	2	a18:3/ 18:4	3	a18:3/16:0	16	a18:3/16:1(3t)	15	18:1/16:0	100	18:2/i18:3	3
a18:3/16:3	16	a18:3/	16:3	11	a18:3/i18:3	8	18:2/16:0	15	a18:3/16:0	9			18:1/ 18:4	2
18:2/16:4	8	a18:3/	16:2	4	18:2/i18:3	12	18:1/16:0	20	18:2/16:1(3t)	41			18:1/i18:3	49
18:2/16:3	2	18:2/	16:3	4	18:1/ 18:4	4	16:0/16:0	49	18:2/16:0	9			18:0/ 18:4	10
a18:3/16:2	1	a18:3/a18:3		4	16:0/ 18:4	15			18:1/16:1(3t)	11			18:0/i18:3	36
18:2/16:2	1	18:2/	16:2	4	18:2/ 18:2	3			18:1/16:0	15				
18:2/16:1	1	18:1/	16:3	3	a18:3/ 18:1	5								
a18:3/16:0	<1	18:1/	16:2	3	18:1/i18:3	6								
18:2/16:0	<1	a18:3/	16:0	18	16:0/i18:3	28								
		18:2/	16:0	25	18:1/ 18:2	3								
		18:1/	16:0	13	16:0/ 18:2	8								
		others		9	others	5								

i18:3 = 18:3(5,9,12), a18:3 = 18:3(9,12,15).

Molecular species were separated by HPLC using an appropriate solvent system for each lipid class. Single molecular species were characterized by the GLC of their constituent fatty acids. All the polar lipids were well resolved into their individual molecular species. It was found that the solvent mixture used for PE also gives good results with SQDG, as shown in Fig. 2. To our knowledge, this is the first separation of SQDG into its constituent species without previous derivatization, thus avoiding loss of material due to the relatively low yield of the derivatization procedure. Although the 16:0/16:0 species does not contain any double bond, it was still detectable, probably due to its high proportion. The percentage of fatty acid combinations in the different lipids are shown in Table 4. MGDG mainly consisted of 18:3(9,12,15)/16:4 and 18:3(9,12,15)/16:3 species. In DGDG, the more saturated species 18:3(9,12,15)/16:0, 18:2/16:0 and 18:1/16:0 predominated. For SQDG, 16:0/16:0 and minor but equal proportions of 18:1/16:0, 18:2/16:0 and 18:3(9,12,15)/16:0 species were typical. In PG, 18:2/16:1(3t) was the major species. In PI, a 18:1/16:0 combination was the only species detectable by HPLC, although additional minor species could be expected from the fatty acid composition (Table 3). DGTS mainly consisted of 16:0/18:3(5,9,12), 16:0/18:4 and 18:2/18:3(5,9,12) combinations. In contrast, PE contained predominantly 18:1/18:3(5,9,12) and 18:0/18:3(5,9,12) species.

Discussion

The distribution of fatty acids between the C-1 and C-2 position of a glycerolipid is generally considered to reflect its biosynthetic origin (Frentzen 1986, Roughan and Slack 1982, 1984): lipids in which the C-2 position is occupied by C_{16} acids are of plastidic (prokaryotic) origin, those with C_{18} acids in that position are of cytoplasmic (eukaryotic) origin. Applying this principle to the lipids of *Chlamydomonas* shows that MGDG, DGDG and SQDG are synthesized exclusively by the chloroplast, whereas only 88% of the PG is produced there. This agrees with the fact that PG is also synthesized by extraplastidic membranes (Marshall and Kates 1972). On the other hand, both DGTS and PE are assumed to be produced almost exclusively by the cytoplasm. The prokaryotic proportions of 14 and 11% of DGTS and PE, respectively, can be explained by the less pronounced fatty acid specificity of the microsomal monoacylglycerol 3-P acyltransferase (Frentzen et al. 1984). The eukaryotic origin of PE also agrees with the finding that this lipid is a marker for the extrachloroplastidial organella (Harwood and Russel 1984, Moore 1982). Moreover, the cytoplasmic origin of DGTS has already been reported for the fern *Adiantum capillus-veneris* by Sato and Furuya (1983).

It is interesting to note that in *Chlamydomonas*, 86% of the PI displays a prokaryotic structure, suggesting that PI is synthesized mostly by the chloroplast, too. Since PI of *Arabidopsis* was found to be mainly eukaryotic (Browse et al. 1986) and PI-synthesizing enzymes could be localized in the endoplasmic reticulum of castor bean endosperm (Moore et al. 1973), the site of synthesis of PI in *Chlamydomonas* could differ from that of higher plants. This hypothesis can be confirmed by showing that purified chloroplasts can synthesize PI.

The difference in fatty acid composition, positional distribution of fatty acids and molecular species composition between the lipids of chloroplasts and those of extraplastidic membranes suggest that in *Chlamydomonas*, both polar lipid assembly as well as fatty acid desaturation, occur separately in each compartment.

It is still not clear how the assembly of polar lipids is coordinated with the desaturation of fatty acids, leading to a complex pattern of molecular species. As a general rule, the formation of polyunsaturated fatty acids starts from 16:0 and 18:1 which are the main products of plastidial fatty acid synthetase and stearoyl desaturase (Stumpf 1984). These two fatty acids are the main substrates for the specific acyl transferases (Frentzen 1986) producing saturated, mono- and diunsaturated glycerolipids which are further desaturated in a lipid-linked process. Such a function has unequivocally been proven for plastidial MGDG (Sato et al. 1986) and cytoplasmic PC (Demandre et al. 1986, Murphy et al. 1985) but is also strongly suggested for other glycerolipids such as PG (Sparace and Mudd 1982), DGTS (Schlapfer and Eichenberger 1983) and PE (Stymne et al. 1987).

These findings argue in favor of a more generalized view of the desaturation process in which every lipid class present in the cell is involved. Such a mechanism would imply that the specificity and activity of the desaturase(s) are greatly influenced by the polar group of the lipid molecule. This concept would explain in a simple way the individual patterns of fatty acids and molecular species observed in the different polar lipids. It would also agree with the findings that in the plant cell, several desaturases exist which introduce double-bonds in particular positions of the acyl chain and/or are specific for lipids with a certain polar group (Browse et al. 1984, 1985).

It seems likely that in *Chlamydomonas*, the biosynthesis of different molecular species starts from saturated (16:0/16:0), monounsaturated (16:0/18:1 or 18:1/16:0 and 18:0/18:1) and diunsaturated (18:1/18:1) diacylglycerol species as parent molecules. In MGDG, a 18:1/16:0 species is probably the starting molecule, although it was not detectable in the HPLC separation. Both C_{16} and C_{18} fatty acids are then sequentially desaturated leading mainly to highly unsaturated species. The same 18:1/16:0 combination may act as parent molecule also for the formation of DGDG. Here, the desatura-

tion, however, leads mainly to species containing 16:0 and minor amounts of species containing 16:2 and 16:3. Such pattern could easily be explained by a DGDG-linked desaturation in which the introduction of double-bonds into C_{16} fatty acids is greatly reduced as compared to the case of MGDG.

A pathway similar to that of galactolipids should also exist for SQDG and PG. For both, a 18:1/16:0 species would act as starting molecule in which the C_{18} fatty acids are predominantly desaturated further. In the case of SQDG, about half of which is formed from 16:0/16:0 species, 16:0 is not desaturated in either position. In PG, only one double-bond is introduced into 16:0 leading to 16:1(3t) which is typical for PG.

Both DGTS and PE apparently are synthesized from 18:1/18:1, 16:0/18:1 and 18:0/18:1 diacylglycerol precursors, all of which are of cytoplasmic origin. The latter two are distributed in different ways: for the synthesis of PE, a 18:0/18:1 species is preferred, whereas the formation of DGTS starts from a 16:0/18:1 combination. In the latter two species, the saturated fatty acids remain unchanged during the subsequent modification, while 18:1 is further desaturated leading mainly to 18:2, 18:3(5,9,12) and 18:4(5,9,12,15). The latter two fatty acids are rather uncommon but have already been detected in Norway spruce (*Picea abies* (L.) Karst.) (Ekman 1980). Also, 18:3 (5t,9c,12c) has bee reported as a major component of *Thalictrum venulosum* (Hitchcock and Nichols 1981).

As for the occurrence of 18:1(11), appreciable amounts have been found in many plants (Hitchcock and Nichols 1971, Johns et al. 1979), although this monoene fatty acid is usually considered to be typical for bacteria and animals (Hitchcock and Nichols 1971). In *Chlamydomonas*, this fatty acid is almost exclusively or predominantly (as in PI) linked to the C-1 position of the glycerol moiety.

The fact that both 18:3(5,9,12) and 18:4(5,9,12,15) are concentrated in DGTS and PE, also sheds light on their formation in *Chlamydomonas*. Little is known about the biosynthesis of 18:3 other than 18:3(9,12,15). In the past, attention had been directed to 18:3(6,9,12). Chavant et al. (1979) suggested that this fatty acid is produced by the cytoplasm. Only recently, Stymne and Stobart (1986) showed that 18:3(6,9,12) is synthesized in the cytoplasm of borage (*Borago officinalis*) seeds in a PC-linked desaturation starting from oleate via linoleate. From these findings and our results, we conclude that 18:3(5,9,12) and 18:4(5,9,12,15) are also formed by the cytoplasm involving a hitherto unknown type of desaturation. Since the 18:3 and 18:4 are found to be linked to the same position of the lipid, one may suggest that the 18:3 is the precursor of 18:4, the Δ^{15}-double-bond being introduced last.

The most striking feature of *Chlamydomonas* is the absence of PC which, in most plants, is a common membrane lipid and which is thought to act as a compulsory intermediate in the formation of triacylglycerols and eukaryotic chloroplast lipids (Roughan and Slack 1982). Thus, no eukaryotic lipids are to be expected if PC is absent. The fact that the chloroplast lipids of *Chlamydomonas* are exclusively prokaryotic is in full accordance with this concept.

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