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Lipid and fatty acids composition of photoautotrophically and heterotrophically grown *Chlamydomonas reinhardtii*

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Abstract

Monogalactosyl diacylglycerol (MGDG), digalactosyl diacylglycerol (DGDG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and sulfoquinovosyl diacylglycerol (SQDG) are the most abundant lipid classes present in both the autotrophically and heterotrophically grown *Chlamydomonas reinhardtii*. However, phosphatidylcholine (PC) and diacylglycerol (*N,N,N*-trimethyl)-homoserine (DGTS) were absent in both alga types. The polyne index B was higher in heterotrophic than photoautotrophic algae, but the unsaturation index was higher in photoautotrophic algae PI, PE and DGDG. The proportion of linolenic acid decreased under heterotrophy with compensatory increases in hexadecadienoic (16 : 3), oleic (18 : 1) and linoleic (18 : 3) acids.

Introduction

The unicellular green alga *C. reinhardtii* is a facultative heterotroph and, when grown in the presence of acetate, synthesizes chlorophyll and photosystem components in the dark (Meek 1974, Guenther *et al.* 1990). The major polar lipids of plant chloroplasts are MGDG, DGDG, SQDG and PG (Nichols 1963). *C. reinhardtii* lipid pattern consists mainly of MGDG, DGDG, SQDG, PG, PE, PI and the betadine lipid DGTS. This pattern depends on the alga strain and the growth conditions. PC is absent in this alga species (Sato and Furuya 1975, Eichenberger 1982, Giroud *et al.* 1988). However, Janero and Barnett (1981) found considerable amounts of PC in

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Abbreviations: ANS - 8-anilinonaphthalin-sulphate; DGDG - digalaktosyl diacylglycerol; DGTS - diacylglycerol (N,N,N-trimethyl)-homoserine; H - heterotrophy; MGDG - monogalactosyl diacylglycerol; P - photoautotrophy; PC - phosphatidylcholine; PE - phosphatidylethanolamine PG - phosphatidylglycerol; PI - phosphatidylinositol; SQDG - sulfoquinovosyl diacylglycerol; UI - index of saturation; Un/S - polyne index = sum of unsaturated fatty acids/sum of the % of mass multiplied by the number of olefinic bonds for each fatty acid in mixture.

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their strains of *C. reinhardtii*. Since it is not easy to obtain clean preperations of *Chlamydomonas* chloroplasts, this study of the lipid composition of *Chlamydomonas* grown under photoautotrophic and heterotrophic conditions was done with whole intact cells.

Materials and methods

Organism and culture conditions: *C. reinhardtii* (11-32a) was obtained from the Sammlung von Algenkulturen, Pflanzenphysiologisches Institut der Universität Göttingen, Germany. The alga was grown photoautotrophically at 25 °C in ammonium/phosphate/trace-metal medium (Sueoka 1960) at pH 7.0 under continuous irradiation by fluorescence lamps [40 μmol(photon) m^{-2} s^{-1}] and continuous bubbling with 4 % CO_2-enriched air. Heterotrophic cells were grown on acetate (0.24 kg m^{-3}) and kept in complete darkness for 4 d before harvesting.

Analysis of lipids: Lipids were extracted from 3 cm^3 culture aliquots by the method of Blight and Dyer (1959). The analysis was carried out according to Sato and Murata (1988). The lipid classes were separated by thin-layer chromatography on precoated silica-gel plates (*5721-Merck*, Darmstadt, Germany) with a mixture of chloroform/methanol/acetic acid/water (85 : 15 : 10 : 3 by vol.) as the mobile phase (Nichols *et al.* 1965). After development, the plates were dried in a stream of CO_2 and the lipid classes were identified using 8-anilinonaphthalin-sulphate (ANS) fluorescence elution and DGDG, MGDG, PC, PE, PI, PG, and SQDG (*Seradary Research Lab*, USA) as standards. The separated lipids in ampoules containing 5 % (m/v) HCl in dry methanol were kept for 2.5 h at 85 °C under N_2. The resultant methyl esters of fatty acids were extracted into n-hexane from the esterification after dilution with an equal volume of water.

Gas chromatography: Methyl esters were analyzed on a gas liquid chromatographic system (*Hewlett Packard 5890 series II*, USA) equipped with a capillary column coated with SP 2330 of 0.25 μm thickness (0.25 mm i.d. × 30 m, *CPS-Li Quardex*, New Haven, CT, U.S.A.). High purity nitrogen was applied at a pressure 230 kPa, hydrogen 100 kPa and oxygen 280 kPa. The dual column system was programmed from 160 to 200 °C to give partial separation of 18:3 at the rate 0.04 °C s^{-1}. The detector and injector temperature were 220 °C. Identification of the peaks was made using a linoleonic standard and by plotting log of relative elution temperature versus the number of carbon atoms (Schmidt and Wynne 1967). To calculate the percentage composition using the *Hewlett-Packard 3396 A integrator*, all peaks emerging between lauric (12:0) and linolenic (18:3) acid were included in calculations.

Results and discussion

Fresh water algae in general contain few fatty acids with more than 3 double bands or 18 carbon atoms (Wood 1974). Table 1 presents the fatty acid composition of total

lipids from *C. reinhardtii* under photoautotrophic and heterotrophic growth conditions. The C12:0 acid was present in this alga strain contrary to previous reports (Eichenberger 1976, Giroud *et al.* 1988, Giroud and Eichenberger 1989). The concentration of the total lipids was remarkably similar in photoautotrophic and heterotrophic alga. Saturated fatty acids, which formed 35.78 % (in photoautotrophic algae) or 36.34 % (in heterotrophic algae) of the total fatty acids in *C. reinhardtii*, consisted almost entirely of C12:0, C14:0, C16:0 and C18:0 acids (Table 1). The polyne index B and the unsaturation index were almost the same and did not depend on the growth conditions of algae.

Table 1. Fatty acid composition of the total lipids from *Chlamydomonas reinhardtii* grown under photoautotrophic and heterotrophic conditions. Un/S, polyne index = sum of unsaturated fatty acids/sum of the % of mass multiplied by the number of olefinic bonds for each fatty acid in mixture.

Fatty acid	Photoautotrophy [mol %]	Heterotrophy [mol %]
12:0	7.48	7.39
14:0	2.46	2.88
16:0	16.26	17.30
16:1	7.70	8.85
16:2	6.49	4.69
16:3	5.21	4.08
18:0	9.22	8.77
$18:1_{cis}$	10.60	11.50
$18:1_{trans}$	12.69	11.33
18:2	11.18	13.78
18:3	10.31	9.41
Un/S	1.79	1.75
UI	112.9	109.1

On the basis of their mobilities during thin-layer chromatography, four major glycerolipid components were identified in *C. reinhardtii*, MGDG, DGDG, SQDG, PG, and two phospholipids, PI and PE (Giroud *et al.* 1988, Giroud and Eichenberger 1989). Hence PC was not present in the lipids of the *C. reinhardtii* strain used in this investigation (Table 1). However, Eichenberger (1976) and Janero and Barrnett (1981) reported the presence of PC in several *Chlamydomonas* strains.

Saturated fatty acids in PI of phototrophic *C. reinhardtii* represented 26.4 % of the total lipids and this value decreased by about 50 %under heterotrophic conditions. The polyne index B in heterotrophic algae increased by about 40 % in PI as compared with the phototrophic alga. However, the unsaturation index decreased in heterotrophic algae which was also observed in PE and DGDG (Fig. 1). The saturated fatty acids identified in the individual lipid classes were C12:0, C14:0, C16:0 and C18:0, the monounsaturated fatty acids C16:1, $C18:1_{cis}$ and $C18:1_{trans}$, the

diunsaturated fatty acids C16:2 and C18:2, and the triunsaturated fatty acids C16:3 and C18:3 (Table 2). In all lipid classes studied, a clear difference in the fatty acid composition between the photoautotrophic and heterotrophic cells was found. The levels of C16:0, C18:3 and C18:1_{trans} were higher in the phototrophic cells than in heterotrophic cells. By contrast, the content of C18:2 acid was higher in heterotrophic cells. All fatty acids were represented in MGDG and PG, however, the C12:0 acid was not present in PI and DGDG in both photoautotrophic and heterotrophic cultures. The C16:3 acid was not represented in PE and SQDG.

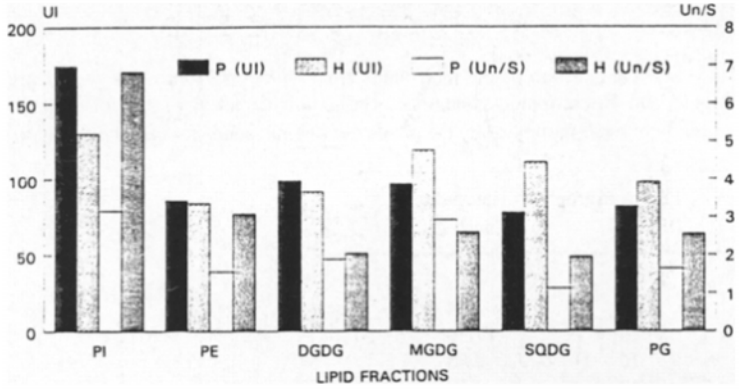

Fig. 1. Polyne index B (Un/S) and index of saturation (UI) of fatty acids of the individual lipid classes isolated from *Chlamydomonas reinhardtii* grown either photoautotrophically (P) or heterotrophically (H) in the dark in the presence of acetate as carbon source. See above mentioned *Abbreviations*.

Virtually all fatty acids found in algal lipids were straight chain molecules containing an even number of carbon atoms. This is a direct consequence of their biosynthesis from acetate by the β-addition. Higher plants characteristically contain α-linolenic acid as a constituents of chloroplast lipids whereas at the other extreme the photosynthetic bacteria possess no polyunsaturated fatty acids. Since the latter are incapable of production of free oxygen during photosynthesis, the presence of linolenic acid might be correlated in some way with the Hill reaction (Erwin and Bloch 1963). This is supported by the observation that in green algae (*Chlorella*) grown in media containing organic compounds, the content of linolenic acid decreases, most markedly in the dark (Nichols 1965). In our experiments the linolenic acid content of PI, PE, DGDG and SQDG decreased from 43, 5.4 and 6.2 %, respectively, in photoautotrophic cells to 3.0, 2.6, 5.0 and 1.6 %, respectively, in heterotrophic cells with compensatory increases in the C16:0, C18:1 and C18:2 acids. Light stimulates fatty acid synthesis in both isolated chloroplasts of higher plants and whole cells of *C. reinhardtii* probably by the same mechanism as in chloroplasts (Picaud *et al.* 1991).

Our *Chlamydomonas* strain did not form C16:4 acids as similarly the one analyzed by Erwin and Bloch (1963) while other strains form the C16:4 acid (Bloch *et al.*

Table 2. Fatty acid composition [mol %] of individual lipid classes isolated from *Chlamydomonas reinhardtii* under photoautotropic (P) and heterotrophic (H) growth conditions.

Fatty acid	PI		PE		DGDG		MGDG		SQDG		PG	
	P	H	P	H	P	H	P	H	P	H	P	H
12:0	-	-	3.07	-	-	-	3.49	4.98	10.22	10.55	1.43	1.39
14:0	-	2.59	3.66	9.72	-	2.06	10.99	3.60	-	4.77	5.89	0.92
16:0	21.4	1.07	27.48	4.35	31.20	30.40	14.52	14.02	25.36	16.00	30.29	19.20
16:1	-	31.00	17.31	37.65	10.08	13.35	4.99	7.71	7.85	7.96	15.51	7.98
16:2	2.56	12.13	-	-	6.37	4.48	4.41	3.15	10.53	20.90	6.28	6.26
16:3	5.08	2.76	-	-	1.98	1.11	4.12	3.32	-	-	0.76	3.32
18:0	5.33	8.96	5.49	10.64	3.66	2.46	7.46	7.78	11.48	2.75	0.75	6.56
$18:1_{cis}$	1.40	14.34	14.05	16.80	17.15	30.58	23.44	12.74	15.31	8.25	23.04	11.60
$18:1_{trans}$	13.67	9.76	8.66	7.75	15.57	11.11	13.26	11.95	-	5.29	7.26	16.40
18:2	8.74	14.39	14.85	10.57	3.63	4.76	5.24	19.55	13.01	21.93	5.14	16.00
18:3	41.75	2.99	5.37	2.61	10.37	4.96	8.08	11.17	6.17	1.55	3.63	9.47
Un/S	2.74	6.92	1.52	3.05	1.87	2.01	2.89	2.59	1.12	1.93	1.61	2.50
UI	178.0	125.0	85.8	83.3	99.9	91.7	97.6	121.0	78.0		81.8	98.3

1967, Eichenberger 1976, Giroud and Eichenberger 1989). In contrast, C16:3 appears to be typical for *C. mundana* (Nichols *et al.* 1967). Our strain formed C12:0 acids which had not been reported in other strains. In other strains (Chuecas and Riley 1969), C18:3 and C20:1 acids predominate instead of C16:4. Thus culture conditions considerably affect the fatty acid composition of microalgae (Orcut and Patterson 1974, Ballantine *et al.* 1979, Borowitzka 1988). This has already been shown for differences in temperature, light and nutrition requirements (Volkman *et al.* 1989, Sriharan *et al.* 1990). Our present results predict compositions that can be expected under different growth conditions. Thus fatty acid synthesis can be affected by culture conditions such as irradiance and carbon source, and the contents of fatty acids in microalgae may vary considerably among species and strains which can be used as a taxonomic characteristic.

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