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Lipids of *Chlamydomonas reinhardtii*. Incorporation of [^{14}C]Acetate, [^{14}C]Palmitate and [^{14}C]Oleate into Different Lipids and Evidence for Lipid-Linked Desaturation of Fatty Acids

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Chlamydomonas reinhardtii, parent strain (ssf), was pulse-labelled with [^{14}C]acetate, [^{14}C]-palmitate or [^{14}C]oleate. Lipids were separated by TLC and HPLC. Radioactivity was measured in each class of lipids and in its fatty acids and molecular species. After 1 hour of incubation with acetate, the label was incorporated mainly into phosphatidylglycerol (PG), diacylglyceryl(*N,N,N*-trimethyl)homoserine (DGTS), digalactosyldiacylglycerol (DGDG) and monogalactosyldiacylglycerol (MGDG). Saturated, monoene and diene fatty acids were strongly labelled. Within 10 hours of incubation in the absence of labelled precursor, the label shifted from monoenes and dienes to trienes and tetraenes. The transfer of radioactivity from mono- to polyunsaturated MGDG and DGDG molecular species suggests a lipid-linked desaturation of the C-1 position (and, in MGDG, also of the C-2 position) of these prokaryotic lipids. In the eukaryotic DGTS, all the species present were labelled simultaneously. On incubation with [^{14}C]-palmitate or [^{14}C]oleate, most of the label appeared in DGTS. Palmitate was immediately incorporated into the polyene species of DGTS, while oleate first appeared in the monoene species and then shifted to the polyene species. From these results it is concluded that, in DGTS, the acyl groups in the C-1 position (mostly 16:0) were rapidly exchanged, while those in the C-2 position (mostly C_{18}) became desaturated to give 18:3(5,9,12) and 18:4(5,9,12,15) acids.

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

Chlamydomonas reinhardtii is characterized by a lipid pattern that consists mainly of MGDG, DGDG, SQDG, PG, PE, PI and the betaine lipid DGTS. PC, however, has been shown to be completely absent from this alga (Giroud et al. 1988). The structure of the plastidial lipids MGDG, DGDG and SQDG was exclusively prokaryotic, in accordance with the absence of PC which, in higher plants, acts as a compulsory intermediate in the formation of plastidial lipids of eukaryotic origin (Roughan and Slack 1982). In a previous report (Giroud et al. 1988) it was shown that, in *Chlamydomonas*, each class of lipids is characterized by an individual pattern of molecular species. MGDG was rich in α18:3, 16:3 and 16:4 fatty acids and mainly consisted of α18:3/16:4 and α18:3/16:3 molecular species. In DGDG, α18:3, 18:1(9), 18:2(9,12) and 16:0 fatty acids were predominant, yielding mainly 18:2/16:0, α18:3/16:0, 18:1/16:0 and α18:3/16:3 molecular species. The major fatty acids of DGTS were 18:3(5,9,12), 18:4(5,9,12,15) and 16:0, leading mainly to 16:0/i18:3, 16:0/18:4 and 18:2/i18:3 combinations. These individual patterns of molecular species gave rise to the suggestion that fatty acids are desaturated in a lipid-linked process in which the different lipids individually act as substrates for

Abbreviations: BHT, butyl hydroxytoluene; DGDG, digalactosyldiacylglycerol; DGTS, diacylglyceryl(*N,N,N*-trimethyl)-homoserine; FID, flame ionization detector; GLC, gas liquid chromatography; HPLC, high-performance liquid chromatography; MGDG, monogalactosyldiacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; SQDG, sulfoquinovosyldiacylglycerol; TLC, thinlayer chromatography; 16:0, hexadecanoic acid; 16:1, hexadecenoic acid; 16:1(3t), 3-*trans*-hexadecenoic acid; 16:2, hexadecadienoic acid; 16:3, hexadecatrienoic acid; 16:3(7,10,13) or a16:3, 7,10,13-hexadecatrienoic acid; 16:3(4,7,10) or b16:3, 4,7,10-hexadecatrienoic acid; 16:4, hexadecatetraenoic aicd; 18:0, octadecanoic acid; 18:1, octadecenoic acid; 18:2 octadecadienoic acid; 18:3, octadecatrienoic acid; 18:3(9,12,15) or α18:3, 9,12,15-octadecatrienoic (α-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.

the desaturase(s). In order to confirm this hypothesis by an analysis of the kinetics of labelling, cells from *Chlamydomonas* were labelled for 1 hour with [^{14}C]-acetate, [^{14}C]palmitate or [^{14}C]oleate and the radioactivity was measured after incubation without label for different periods of time in each class of lipid and in the associated fatty acids and molecular species.

Material and Methods

Plant material—Chlamydomonas reinhardtii 137c arg-2 mt$^+$ from (The Culture Centre of Algae and Protozoa, Cambridge, U.K.) was cultivated autotrophically under 10,000 lux of continuous fluorescent light for 2.5 days at 26–28°C. The nutrient medium was medium I of Sager and Granick 1954, supplemented with 0.47 mM arginine-HCl.

Incubation conditions—Cells in the middle to late logarithmic phase of growth (10^6 cells/ml, 2.5 days old) were harvested by centrifugation at about $200 \times g$ for 2.5 min (Sorvall RC 58 rotor GSA) and resuspended in fresh medium with adjustment of the concentration of cells to 10^8 cells/ml. After addition of 2 mM *sn*-glycerol-3-phosphate, the suspension of cells was incubated for 1 hour in the light with either [2-^{14}C]acetate (58 mCi/mmol, 20 μCi/10^9 cells), [1-^{14}C]palmitate (54 mCi/mmol, 5 μCi/10^9 cells) or [1-^{14}C]oleate (56 mCi/mmol, 5 μCi/10^9 cells). After a 100-fold dilution with fresh medium I, the suspension was kept under light conditions for another 23 hours.

Analysis of lipids—Samples were withdrawn after various incubation times and cells were collected by centrifugation. The lipids were extracted with methanol which contained 0.05% BHT as an antioxidant and spotted on precoated silica gel plates (Merck 5715) with a sample applicator (Linomat III, CAMAG, Muttenz, Switzerland). After development of the plates with chloroform/methanol/acetic acid/water (85 : 15 : 10 : 3, by vol.) (Nichols et al. 1965) the radioactive spots were localized by scanning the plate with a Bioscan System 200 Imaging Scanner (Bioscan, Washington, D.C.) linked to an IBM XT computer. The lipid bands were detected under UV light (366 nm) after spraying of the plate with 2',7'-dichlorofluorescein. After addition of 2 ml methanol and 5 ml 0.7% (w/v) butyl-PBD (Ciba-Geigy, Basel, Switzerland) in toluene, the radioactivity was monitored in a MR-300 Liquid Scintillation Counter (Kontron, Switzerland). Pigments were bleached with Cl_2, if required. The counting efficiency was 85%.

Analysis of fatty acids—Lipids (2 mg per plate) were separated by 2-dimensional TLC on silica gel (Merck 5715) plates developed with chloroform/methanol/water (65 : 25 : 4, by vol.) in the first dimension and chloroform/methanol/isopropylamine/conc. NH_3 (65 : 30 : 0.5 : 5, by vol.) in the second dimension. After elution with methanol that contained 0.05% BHT, the lipids were purified by partition between 2 ml of 1% NaCl and 5 ml chloroform/methanol (2 : 1, v/v), dried under N_2 and then hydrolyzed in the presence of 500 μl of KOH/H_2O/ethanol (1 : 2 : 20, w/v/v) at 70°C for 30 min. After dilution with 5 ml H_2O, the nonsaponifiable material was extracted with 53 ml hexane/diethylether (1 : 1, v/v) and the aqueous phase acidified with 100 μl conc. HCl/H_2O (1 : 1, v/v). The free fatty acids were extracted with hexane/diethyl ether and the solvent evaporated with N_2. After addition of 20 μl of 0.1% BHT in methanol and drying under N_2, the fatty acids were converted to their phenacyl esters by the procedure of Borch (1975). The residue was combined with 50 μl of freshly distilled triethylamine in acetone (10 mg/ml) and 50 μl of recrystalized phenacylbromide in acetone (12 mg/ml) and kept overnight at room temperature. The phenacyl esters were dried, redissolved in 30 μl acetonitrile and separated by reversed-phase HPLC on a Perkin-Elmer Series 10 Liquid Chromatograph (Perkin-Elmer, Norwalk, Conn., U.S.A.). A 20-μl solution of phenacyl esters was loaded on a column (250 × 4 mm) that contained Nucleosil 5 C 18 (Knauer, Homburg, F.R.G.). Acetonitrile and water were used as eluent (10 min of 68% CH_3CN, and then from 68% to 92% CH_3CN in 48 min) and the flow rate was 1.5 ml/min. The phenacyl esters were detected at 242 nm with a Perkin-Elmer LC-75 detector connected to a LCI-100 Integrator. The radioactivity was measured after UV detection with a Berthold LB 505 HPLC radioactivity Monitor equipped with a 200 μl GT200U4 solid scintillation cell (Berthold, München, F.R.G.) with a counting efficiency of 50%. Peaks were integrated with a Shimadzu Chromatopac C-R3A Integrator (Shimadzu, Tokyo, Japan). For the identifications, fractions corresponding to single peaks were collected, taken to dryness under N_2 in the presence of BHT and then converted to methyl esters by transesterification with sodium methoxide (Thies 1971). Methyl esters were analyzed by capillary GLC as described by Giroud et al. (1988).

Analysis of molecular species—MGDG, DGDG, DGTS and PE were eluted from the TLC plates and then purified by HPLC on a column (250 × 4 mm) of Spherisorb S3 NH_2 (Knauer) with acetonitrile and water as eluents. The gradient was from 100% to 68% acetonitrile in 40 min and the flow rate was 1.5 ml/min. The lipids were detected at 210 nm, collected and resolved into their constituent molecular species on a reversed-phase column (250 × 4 mm) of Spherisorb S3 ODS II (Knauer). MGDG and DGDG were eluted by a linear gradient of 94% (v/v) methanol/water (A) and methanol (B). The flow rate was 1.1 ml/min and the gradient was from 100% A to 100% B in 50 min. For DGTS and PE, an isocratic elution was performed using a mixture of methanol/water/acetonitrile (94 : 3.5 : 2.5, by vol.) that contained 20 mM choline-HCl. The flow rate was 1.8 ml/min for DGTS and 1.4 ml/min for PE. The

effluent was monitored at 202 nm and the radioactivity measured with the Berthold LB 505 HPLC counter.

Results

In order to investigate the kinetics of labelling of the different lipids, cells were incubated with [2-^{14}C]acetate and, after a 100-fold dilution, kept under light conditions for another 23 hours. During the pulse, the precursor was absorbed almost completely by the cells at a linear rate. Of the total radioactivity, 4–8% was incorporated into the polar lipids during the experiments. At the end of the pulse, PG and DGTS were the most strongly labelled lipids, as shown in Fig. 1.

During the chase, the radioactivity accumulated essentially in MGDG, DGTS and DGDG, which are the major membrane lipids of *Chlamydomonas* cells. Minor amounts of label were found in SQDG, PE and PI. It is appropriate to mention that, generally, the major portion of the acetate label was incorporated into the fatty acids and that the distribution of radioactivity between acyl groups and polar groups remained almost constant during the whole experiment. When the cells were incubated with [2-^{14}C]acetate at a concentration greater than 10^7 cells/ml, the label was incorporated mainly into saturated, monoene and diene fatty acids, and only minor amounts appeared in the triene and tetraene acids, even if the duration of the incubation was extended to 24 hours (results not shown). In contrast, when a concentration of 10^6 cells/ml was used, the radioactivity was also incorporated into polyenoic fatty acids. In order to demonstrate the kinetics of labelling of individual fatty acids, phenacyl esters were prepared and separated by reversed-phase HPLC. Except for 16:0 and 18:1 fatty acid esters, the single constituents were well separated by this procedure, as shown in Fig. 2. With picolinyl esters instead of phenacyl esters, the resolution could not be improved.

The kinetics of labelling of individual fatty acids and molecular species of MGDG are shown in Fig. 3.

After 1 hour of incubation, most of the label was found in 16:0 and C_{16} and C_{18} monoene and diene fatty acids. Correspondingly, the molecular species containing these fatty acids were also strongly labelled. During the subsequent 23 hours, the radioactivity shifted from the less to the more unsaturated fatty acids and molecular species and finally accumulated in the α18:3/16:4 major species of MGDG. It is noteworthy that, of the two 16:3 isomers, 16:3(7,10,13) was strongly labelled after a short time, while a small amount of label appeared only belatedly in 16:3(4,7,10). This result clearly indicates that the 16:4 fatty acid is formed from the 16:2 acid via the 16:3(7,10,13) acid but not via the 16:3(4,7,10) isomer. The kinetics of labelling of the different molecular species suggested that the rate of desaturation of C_{16} fatty acids in the C-2 position was different from that of the C_{18} fatty acids, which are concentrated in the C-1 position. The C_{16} acids were more rapidly desaturated, as indicated by the relatively high level of label in the 18:1/16:3 and 18:1/16:4 species. In contrast, no radioactivity was found in the 18:2/16:0 and α18:3/16:0 species.

The kinetics of labelling of DGDG were quite different from those of MGDG, as shown in Fig. 4.

Once again, at the end of the pulse, the label was found mainly in 16:0 and in monoene and diene fatty acids. During the experiment, the label was transferred from 18:1 to α18:3 acid, and, to a much lesser extent, from 16:0 to a16:3 and 16:4 acids. This result indicates that the 18:1 acid, which is mainly located at the C-1 position, is

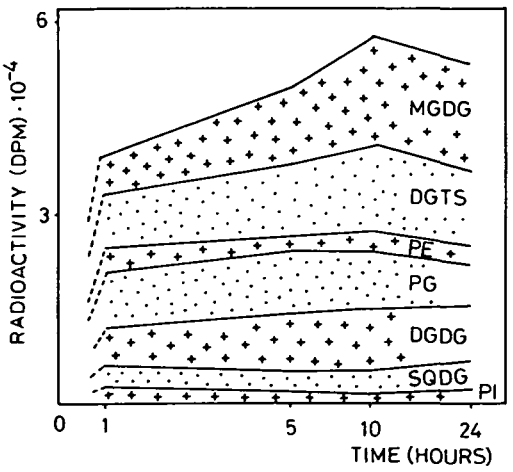

Fig. 1 Incorporation of [2-^{14}C]acetate into lipids of *Chlamydomonas reinhardtii*. Pulse: 1 hour, 20 μCi/10^9 cells, 10^8 cells/ml; chase: 10^6 cells/ml.

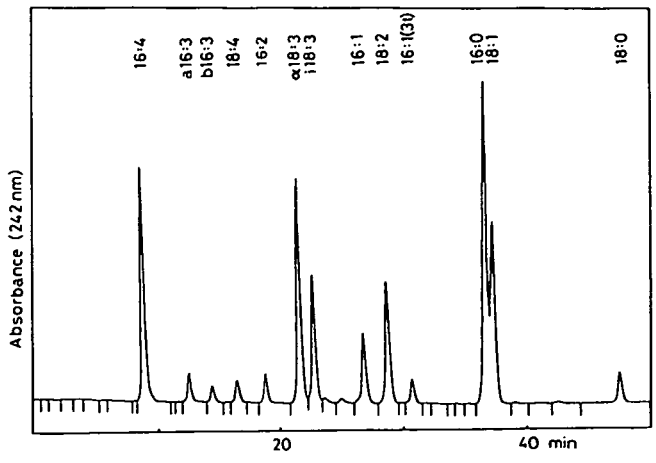

Fig. 2 Separation by reversed-phase HPLC of fatty acid phenacyl esters from total lipids of *Chlamydomonas reinhardtii*. For conditions see Material and Methods.

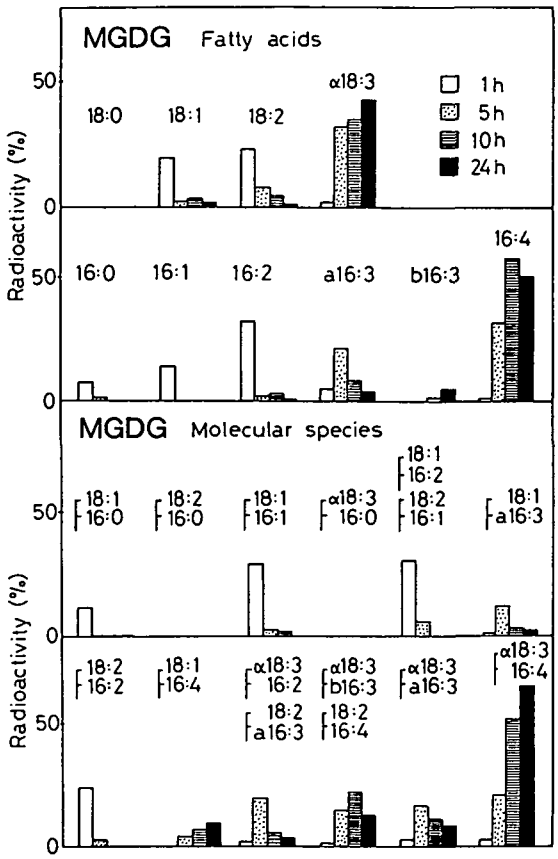

Fig. 3 Incorporation of [2-^{14}C]acetate into fatty acids and molecular species of MGDG from *Chlamydomonas reinhardtii*. a16:3 = 16:3(7,10,13); b16:3 = 16:3(4,7,10).

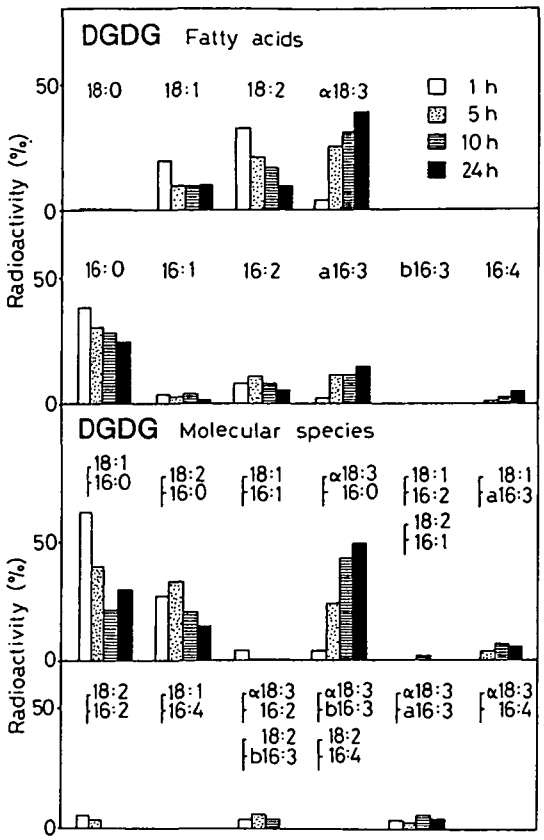

Fig. 4 Incorporation of [2-^{14}C]acetate into fatty acids and molecular species of DGDG from *Chlamydomonas reinhardtii*. a16:3 = 16:3(7,10,13); b16:3 = 16:3(4,7,10).

promptly desaturated to give α18:3 acid, while the 16:0 acid (mainly located at the C-2 position) is only weakly desaturated and, even then, not further than a16:3. No radioactivity appeared in the other 16:3 isomer, suggesting that the latter was formed on MGDG only.

With respect to the molecular species, the 18:1/16:0 and 18:2/16:0 combinations were heavily labelled at the beginning of the incubation. During the experiment, the radioactivity in these species appeared to be transferred to the α18:3/16:0 species, again suggesting that, in DGDG, C_{18} fatty acids were preferentially desaturated. The changes with time in the distribution of radioactivity between the different labelled fatty acids of DGTS exhibit some interesting differences to those of the two galactolipids, as shown in Fig. 5.

In the C_{16} series, no radioactivity was transferred from saturated to unsaturated fatty acids, and the relative amount of the label in 16:0 acid remained constant during the whole experimental period. In contrast, the C_{18} acids were rapidly desaturated, with the label shifting from 18:1 to 18:3(5,9,12) and also, to a lesser extent, to 18:4 acid. A minor amount of radioactivity was also found in α18:3 acid. Analysis of the molecular species of DGTS showed that almost all the combinations present were already labelled near the beginning of the experiment, and that there was no clear evidence of the transfer of radioactivity from the less to the more unsaturated molecular species. After 1 hour, most of the label was already found in polyunsaturated species. In order to elucidate more thoroughly the pathways that lead to the unsaturated fatty acids of DGTS, cells were incubated with [1-^{14}C]palmitate or [1-^{14}C]oleate under the same conditions as the incubation with radiolabelled acetate.

In order to ascertain the positional distribution of the label after 24 hours of chase, the lipids were hydrolyzed with *Rhizopus* lipase and the radioactivity was measured in the free fatty acid (C-1 position) and the lyso compound (C-2 position). In MGDG and DGDG (both prokaryotic), 16:0 was incorporated mainly into the 2-position, while in (eukaryotic) DGTS, the same precursor was incorporated predominantly into the 1-position, as shown in Fig. 6. The 18:1 acid, by contrast, was incorporated mainly into the 1-

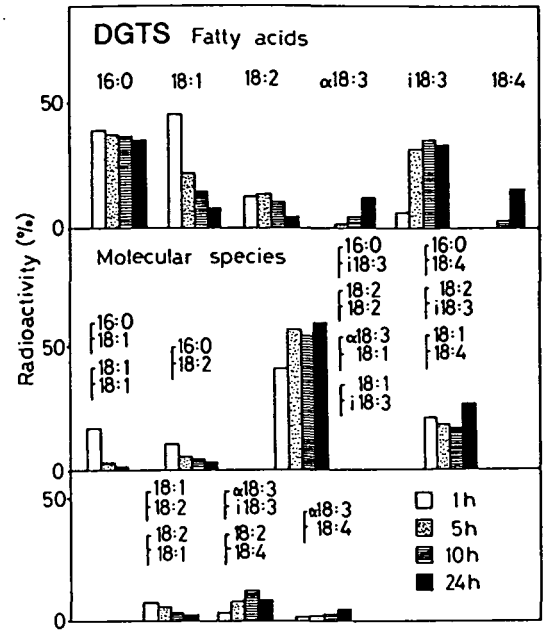

Fig. 5 Incorporation of [2-^{14}C]acetate into fatty acids and molecular species of DGTS from *Chlamydomonas reinhardtii*. i18:3 = 18:3(5,9,12).

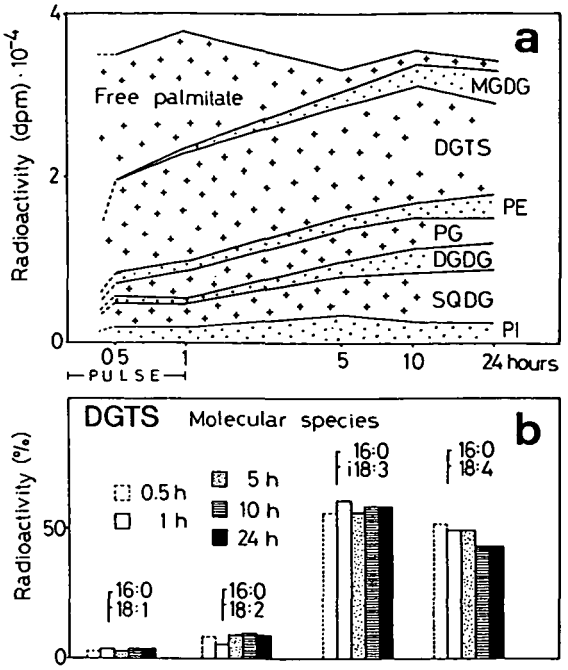

Fig. 7 Incorporation of [1-^{14}C]palmitate into a) different lipids, b) molecular species of DGTS from *Chlamydomonas reinhardtii*. i18:3 = 18:3(5,9,12).

position of both MGDG and DGDG, and into the 2-position of DGTS.

With [1-^{14}C]palmitate as substrate, after 30 min, most of the precursor was absorbed by the cells, but only half of the total radioactivity was found in the polar lipid fraction, as shown in Fig. 7a.

After the end of the pulse, the label in the polar lipid fraction rapidly increased at the expense of free palmitate. Most of the label appeared in DGTS and minor amounts in PG and SQDG. It is noteworthy that palmitate was incorporated immediately into the polyunsaturated molecular species which consisted mainly of 16:0/i18:3 and 16:0/18:4

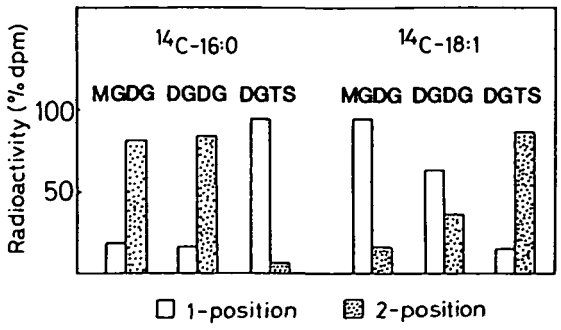

Fig. 6 Distribution of radioactivity among the C-1 and C-2 positions after incubation with [1-^{14}C]palmitate or [1-^{14}C]oleate (1 hour pulse, 23 hours chase).

combinations, as shown in Fig. 7b. In these species, the amount of radioactivity remained almost unchanged during the experiment, while the mono- and diunsaturated species were only weakly labelled.

When [1-^{14}C]oleate was used as a precursor, once again the major portion of the label was incorporated into DGTS, as shown in Fig. 8a.

Toward the end of the incubation period, the label also appeared in MGDG, PE and DGDG. It is noteworthy that the label from oleate appeared first in the molecular species of DGTS that contained 18:1 and 18:2 fatty acids and then shifted to the polyunsaturated species which consisted mainly of 16:0/i18:3, 18:1/i18:3 and 18:1/18:4 combinations. This result indicates that the processing by the cell of 16:0 acid was different from that of 18:1 acid and suggests that the acyl groups in the C-1 position (mainly 16:0) were rapidly exchanged, while those in the C-2 position (mostly C_{18}) became desaturated to give 18:3(5,9,12) and 18:4(5,9,12,15) acids. During the final hours of the experiment, there was a slight decrease in the total radioactivity in the polar lipid fraction, indicating that some turnover of the fatty acids had occurred.

Similar results (not shown here) were also obtained with PE which is, like DGTS, of cytoplasmic origin. After 1 hour of incubation with [2-^{14}C]acetate, the label was concentrated in 18:0 and 18:1 acids. Over the course of the experiment, the relative amount of radioactivity in these two

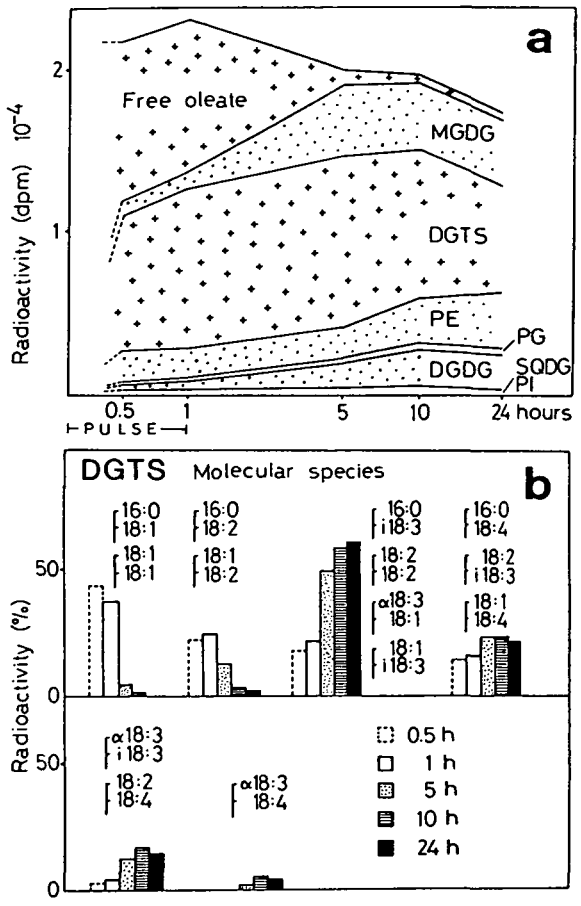

Fig. 8 Incorporation of [1-^{14}C]oleate into a) different lipids, b) molecular species of DGTS from *Chlamydomonas reinhardtii*. i18:3 = 18:3(5,9,12).

fatty acids decreased rapidly, while the label in the 18:3(5,9,12) isomer increased rapidly. However, the α18:3 acid was not labelled, and the 18:4 acid was only weakly labelled, unlike the situation for the same fatty acids in DGTS. It is appropriate to mention also that, after incubation for 1 hour with [2-^{14}C]acetate, the distribution of radioactivity between the different molecular species remained unchanged during the chase period. By contrast, with [1-^{14}C]oleate as precursor, the label first appeared in two species that were tentatively identified as 18:0/18:2 and 18:1/18:2 combinations, and was then transferred to the polyunsaturated species, which consisted mainly of 18:0/i18:3, 18:1/i18:3 and 18:1/18:4 species. These results indicate that, in PE, the fatty acids in the C-1 position (mainly 18:0 and 18:1) were exchanged, while those in the C-2 position (mainly C_{18}) were desaturated. Thus, it appears that, in PE as in DGTS, fatty acids are processed in a manner that is dependent on their position on the glycerol moiety.

Information was also obtained on the fate of the 16:0 acid of PG and SQDG. When *Chlamydomonas* cells were supplied with [2-^{14}C]acetate, palmitate was initially the most strongly labelled fatty acid in these lipids (results not shown). During the subsequent chase, a rapid decrease of the label in 16:0 and a dramatic increase in label in 16:1(3t) acid in PG was observed, indicative of a specific involvement of this lipid in the formation of 16:1(3t) acid from 16:0 acid. In contrast, the label in the 16:0 acid of SQDG remained unchanged. The label in the 18:1 acid of these two lipids was transferred to the 18:2 acid and finally to the α18:3 acid, indicating a sequential desaturation of 18:1 to α18:3 acid.

In PI, only 16:0 and 18:1 acids were labelled. In the course of the experiment, the amount of label in palmitate increased continuously whilst the amount in oleate rapidly diminished. The kinetics of labelling and the biosynthesis of PI in *Chlamydomonas* are the focus of further experiments.

Discussion

When the cells of *Chlamydomonas* were incubated with [2-^{14}C]acetate at a concentration of 10^6 cells/ml, the incorporation of label into polyenoic fatty acids was increased as compared to incorporations during incubations at higher concentrations of cells. This result indicates that the desaturation which leads to formation of tri- and tetraenoic fatty acids is inhibited in concentrated suspensions of cells. Although the reason for this inhibition is not known, it offers a simple method for accumulation of the less unsaturated precursors of polyunsaturated fatty acids, which are otherwise only hardly detectable. Mutants that are unable to perform certain desaturation steps (Norman and St. John 1986) and inhibition by herbicides (Norman and St. John 1987) have been used in alternative strategies to achieve the same end.

In *Chlamydomonas*, a transfer of radioactivity from less to more unsaturated molecular species was observed in MGDG and, in a different way, in DGDG, which is thought to be synthesized by a transfer of a galactosyl moiety from one molecule of MGDG to another (Van Besouw and Wintermans 1978). The biosynthetic interrelationship between MGDG and DGDG and between different molecular species in *Chlamydomonas* is summarized in Fig. 9.

It is suggested that the biosynthesis of both galactolipids started from the 18:1/16:0 species of MGDG, which was labelled first. With time, however, the radioactivity was channeled differently in the two lipids. In MGDG, the label accumulated in the α18:3/16:4 species, while in DGDG it was concentrated in the α18:3/16:0 species. This result indicates that mainly the 18:1/16:0 molecular species from MGDG were used for the formation of DGDG and that the major part of 16:0 of DGDG remained unchanged. This concept is in accordance with the hypoth-

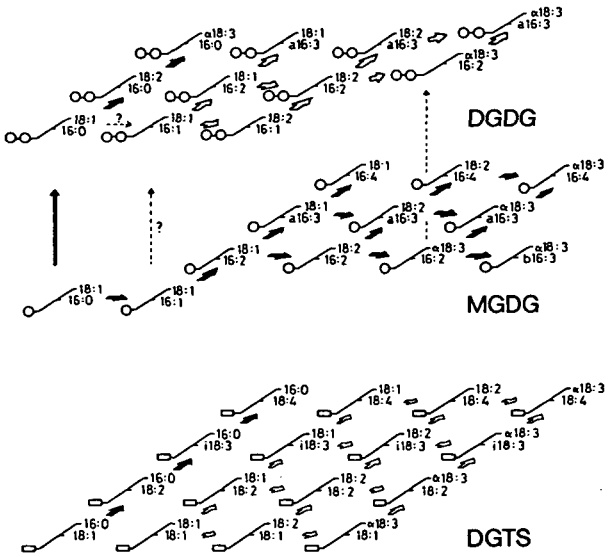

Fig. 9 Pathways of the lipid-linked desaturation of fatty acids in MGDG, DGDG and DGTS of *Chlamydomonas reinhardtii*. a16:3 = 16:3(7,10,13); b16:3 = 16:3(4,7,10); i18:3 = 18:3(5,9,12).

esis that the n-9 desaturase is not active with the C_{16} fatty acids of DGDG (Frentzen 1986). Although the transformation to DGDG of 18:1/16:1 MGDG species cannot be excluded, the small amount of label in *a*18:3/16:3 species of DGDG can easily be explained by a limited galactosylation of a polyene species of MGDG. The galactosylation of polyene species up to hexaenes has also been observed in chloroplasts from peas (Siebertz and Heinz 1977) and from spinach (Heemskerk 1986). In these cases, the polyene species of MGDG are actually the preferred substrates of the galactosyltransferase. As a consequence of these specificities, in MGDG of *Chlamydomonas*, fatty acids are desaturated at both the C-1 and C-2 position, while in DGDG, the desaturation occurs predominantly at the C-1 position.

In the case of the cytoplasmic lipid DGTS, the situation in more complex than that for the galactolipids. On the one hand, radioactivity from [^{14}C]acetate and [^{14}C]-palmitate rapidly appeared in all kinds of 16:0-containing molecular species of DGTS, even if the desaturation was blocked at the level of dienes by a high concentration of cells in the incubation mixture. In this case, no shift of radioactivity was observed from less to more unsaturated species. On the other hand, [^{14}C]oleate first appeared in monoene and diene species and only later also in polyene species. From these results we conclude that in DGTS, the fatty acids in the C-1 position, which mainly consist of 16:0 acid, are rapidly exchanged between the lipid and possibly acyl-CoA. Such an acyl exchange for DGTS was previously postulated and its existence was strongly supported in double-labelling experiments by Schlapfer and Eichenberger (1983). In contrast, the fatty acids in the C-2 position of DGTS, which mainly consist of 18:1 acid, are not exchanged but are desaturated in a lipid-linked process to give 18:3(5,9,12) and 18:4(5,9,12,15) acids (Fig. 9).

A similar exchange has to be assumed also in the case of PE, of which the fatty acids at the C-1 position are exchanged, while those at the C-2 position are desaturated. The significance of this process in *Chlamydomonas* is not known. A similar acyl exchange has been demonstrated with microsomes from safflower cotyledons, in which unsaturated fatty acids are exchanged between the C-2 position of PC and acyl-CoA which, in turn, is used for the synthesis of unsaturated triacylglycerols (Stymne and Stobart 1984). This mechanism is unlikely to occur in *Chlamydomonas*, since this alga does not produce triacylglycerols in significant amounts.

Since labelled 16:0 and 18:1 fatty acids were mainly incorporated into DGTS, exogenous fatty acids seem to be used predominantly for the production of this lipid, while only minor amounts of these fatty acids are incorporated into MGDG, DGDG, SQDG and PG, all of which are purely prokaryotic in this alga. It is noteworthy that the incorporation of exogenous 16:0 and 18:1 fatty acids into prokaryotic lipids has also been reported for *Dunaliella* (Norman et al. 1985). In this alga, whose plastidial lipids are also prokaryotic (Lynch et al. 1983), palmitic acid is incorporated mainly into the C-2 position of PG (Norman and Thompson 1985). These results indicate that, in *Chlamydomonas* and *Dunaliella*, exogenously administered fatty acids may be transferred to the chloroplast, where they enter the prokaryotic pathway. In spinach leaves, by contrast, exogenous 16:0 acid is incorporated into prokaryotic MGDG only via eukaryotic MGDG species and a redistribution of acyl groups (Thompson et al. 1986, Roughan et al. 1987). These results may indicate that lower organisms metabolize exogenous fatty acids in a way which is different from that of higher plants.

Our results clearly demonstrate that in *Chlamydomonas*, the desaturation of fatty acids occurs in different ways in each cell compartment, since the polyunsaturated fatty acids produced by the ER and attached to DGTS and PE are structurally different from those of the glycolipids and PG, which are produced by the plastids. The former consist of i18:3 and 18:4 acids, while the latter are mainly 16:4 and *a*18:3, and 16:1(3t) acids. DGTS is suggested to be the main substrate for the cytoplasmic desaturase(s), substituting for PC at least in this function. The kinetics of labelling of molecular species strongly support the generally held view of the lipid-linked desaturation process, in which every lipid is involved individually. Uptil now, such a role has only been demonstrated properly for MGDG (Sato et al. 1986) and for PC (Demandre et al. 1986, Murphy et al. 1985).

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