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Radiolabelling Studies on the Lipid Metabolism in the Marine Brown Alga *Dictyopteris membranacea*

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The lipid metabolism of the marine brown alga *D. membranacea* was investigated using [2-^{14}C]acetate, [1-^{14}C]myristate, [1-^{14}C]oleate and [1-^{14}C]arachidonate as precursors. On incubation with [2-^{14}C]acetate, 18:1 and 16:0 were the main products formed by de novo synthesis and incorporated into polar lipids. With all the exogenous substrates used, DGTA was strongly labelled and the subsequent rapid turnover of radioactivity suggested a key role for this lipid in the redistribution of acyl chains and most likely also in the biosynthesis of the eukaryotic galactolipids produced in the absence of PC. In the glycolipids a continuous accumulation of radioactivity was observed with all the substrates used. The labelling kinetics of molecular species of MGDG suggested the desaturation of 18:1 to 18:4 and of 20:4 (n-6) to 20:5 (n-3) acids on this lipid. Both PG and PE were primary acceptors of de novo synthesized fatty acids and exogenous [1-^{14}C]oleate, but no evidence exists for a further processing of acyl chains on these lipids. TAG, although strongly labelled with all exogenous [1-^{14}C]acids, was not labelled when [2-^{14}C]acetate was used as a precursor indicating the flux of endogenous fatty acids to be different of that of exogenously supplied fatty acids.

Key words: DGTA — *Dictyopteris membranacea* — Eicosapentaenoic acid — Galactolipids — Metabolism — Phaeophyceae.

In a preceding paper (Hofmann and Eichenberger 1997), the lipid and fatty acid composition of the brown alga *Dictyopteris membranacea* was analysed in detail. This alga clearly shows some of the characteristics of brown algae (Dembitsky 1996, Harwood and Jones 1989). It contains PUFAs like 18:4, 20:4 and 20:5 which are not common constituents of higher plants (Gurr and Harwood 1991). Considering polar lipids, *D. membranacea* contains very high proportions of glycolipids including galactolipids predominantly of the eukaryotic type. Phospholipids are present in minor amounts only and among them, PC is not detectable. This alga, however, contains the betaine lipid DGTA. The occurrence of this lipid and of PC in brown algae was shown to reflect their taxonomy (Araki et al. 1991, Eichenberger et al. 1993). Three groups could be distinguished containing either DGTA only, PC only or both these lipids. The orders Dictyotales, Durvillaeales and Fucales contained DGTA only. As a member of the Dictyotales, *D. membranacea* is therefore a suitable organism for studying the metabolic role of the betaine lipid DGTA. This lipid was highly labelled from [1-^{14}C]acetate in *Fucus serratus* (Smith and Harwood 1984), *Ascophyllum nodosum* and *F. vesiculosus* (Jones and Harwood 1993) indicating an active role of DGTA in the metabolism of these algae. In this context it is interesting to note that DGTS, which is a structural isomer of DGTA, was shown to act as the primary acceptor of exogenous 18:1 acid, as the substrate of 18:1 to 18:2 desaturation and as a donor of 18:2 acid for further desaturation or elongation reactions in the chrysophyte alga *Ochromonas danica* (Vogel and Eichenberger 1992). In the green alga *Chlamydomonas reinhardtii*, DGTS was found to be involved in the lipid-linked desaturation of fatty acids (Giroud and Eichenberger 1989).

Another characteristic feature of *D. membranacea* is the absence of the widely distributed phospholipid PC which is an essential intermediate in the formation of the eukaryotic type chloroplast lipids in higher plants (Ohlrogge and Browse 1995, Roughan and Slack 1982). According to this concept, eukaryotic diglyceride moieties with C_{18} fatty acids at the *sn*-2 position are synthesised by cytoplasmic membranes and converted to PC. The eukaryotic DAG moieties of PC are then translocated to the chloroplast and used for the assembly of the plastidial glycolipids. The high level of eukaryotic galactolipids in *D. membranacea* raises the question about the origin of these compounds in the absence of detectable amounts of PC. In the present work the incorporation of different radiolabelled fatty acids and fatty acid precursors into the lipids of *D. membranacea* and their further processing was examined. In order to gain some information about the metabolic role of DGTA and the assembly of MGDG, the

Abbreviations: Butyl-PBD, 2-(4′-*t*-butylphenyl)-5-(4″-biphenylyl)-1,3,4-oxadiazol; DAG, diacylglycerol; DGCC, diacylglyceryl-carboxyhydroxymethylcholine; DGDG, digalactosyldiacylglycerol; DGTA, diacylglycerylhydroxymethyl-*N,N,N*-trimethyl-*β*-alanin; DGTS, diacylglyceryl-*N,N,N*-trimethylhomoserine; GLC, gas liquid chromatography; RP-HPLC, reversed-phase high-performance liquid chromatography; MGDG, monogalactosyldiacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PHEG, phosphatidyl-*O*-[*N*-(2-hydroxyethyl)glycine]; PI, phosphatidylinositol; PUFAs, polyunsaturated fatty acids; SQDG, sulfoquinovosyldiacylglycerol; TAG, triacylglycerol; TLC, thin-layer chromatography.

labelling kinetics within the molecular species of these lipids were analysed.

Materials and Methods

Plant material—A unialgal clonal culture of a *Dictyopteris membranacea* (Stackhouse) Batters tetrasporophyte (collected in Villefranche-sur-mer, France, Mediterranean Sea) was obtained from Prof. D.G. Müller, Faculty of Biology, University of Konstanz, Germany. The alga was cultivated in glass dishes with 50 ml of culture medium prepared from autoclaved natural seawater (North Sea, salinity 28‰) supplemented with PES as specified by Starr and Zeikus (1993). The algae were grown under a light/dark cycle of 12/12 h at 18°C under white fluorescent light (60 μE m^{-2} s^{-1}).

Radioactive compounds—[2-^{14}C]acetate (99.9 MBq $mmol^{-1}$), [1-^{14}C]oleate (1.9 GBq $mmol^{-1}$) and [1-^{14}C]arachidonate (2.0 GBq $mmol^{-1}$) were purchased from New England Nuclear and [1-^{14}C]myristate (2.0 GBq $mmol^{-1}$) from Amersham International.

Incubation conditions—In a standard assay, the algae (~2–3 g fr wt) were incubated in a 50 ml Erlenmeyer flask with 5 ml culture medium containing 0.005% Tween 80 and labelled substrate which was suspended by sonication. Of acetate, myristate and oleate, 185 kBq, and of arachidonate 111 kBq were added. The incubation was carried out under constant fluorescent light with shaking at 20°C for 1 h. At the end of the pulse, the medium was removed and the algae rinsed three times with seawater. Equal portions were distributed among 4 glass dishes with 50 ml culture medium supplied with 0.5 mM acetate. The algae were then grown under normal conditions up to 6 d and aliquots were taken after different times of chase.

Analysis of lipids—After the extraction of different chase samples with hot methanol, total lipids were separated on precoated silica gel plates (Merck 5715) with chloroform/methanol/water (65 : 25 : 4, by vol.) in the 1st dimension and chloroform/methanol/isopropylamine/conc. ammonia (65 : 35 : 0.5 : 5, by vol.) in the 2nd dimension. Spots were detected under UV light (366 nm) after spraying with 2',7'-dichlorofluorescein. TAG was separated from polar lipids and pigments by TLC with chloroform/methanol (50 : 1, by vol.). Radioactive spots on TLC plates were localized either with a System 200 Imaging Scanner (Bioscan, Washington, D.C.) or by autoradiography with Hyper film MP (Amersham). For the quantification of radioactivity, single spots were scraped off and measured on a Betamatic V liquid scintillation counter (Kontron, Switzerland) after addition of 2 ml of methanol and 5 ml of 0.7% (w/v) butyl-PBD (Glaser) in toluene.

Analysis of fatty acids—Labelled fatty acids of total and single lipids were obtained by alkaline hydrolysis with KOH/water/ethanol (1 : 2 : 20, w/v/v) for 30 min at 70°C. The free fatty acids were then dried under N_2 and converted into their phenacylesters according to Borch (1975). The dried residues were combined with 100 μl distilled triethylamine in acetone (10 mg ml^{-1}) and 100 μl of recrystalized phenacylbromide in acetone (12 mg ml^{-1}) and allowed to react at 50°C for 2 h. The dried phenacylesters were redissolved in 40 μl acetone and separated by RP-HPLC. For the separation, a Shimadzu LC-6A liquid chromatograph with a Nucleosil 100-5 C_{18} (250 × 4 mm, Macherey Nagel) column was used. The solvents were acetonitrile (A) and acetonitrile/water (6 : 4, by vol.) (B). The gradient was from 20% to 65% A in 60 min with a flow rate of 1.5 ml min^{-1}, followed by 80% A for 20 min with a flow rate of 2 ml min^{-1}. Detection was at 242 nm with a Shimadzu SPD-6A spectrometer. Single peaks were collected and the fatty acids transesterified (Thies 1971) and identified by GLC as described before (Hofmann and Eichenberger 1997). Single fractions of the HPLC run were collected and their radioactivity determined by liquid scintillation counting after addition of 4 ml of methanol and 5 ml of 0.7% (w/v) butyl-PBD (Glaser) in toluene.

Analysis of molecular species of DGTA and MGDG—The molecular species of labelled lipids were separated by RP-HPLC using the equipment described above. For DGTA, the solvents were methanol/water/acetonitrile (80 : 12 : 8, by vol.) (A) and methanol/water/acetonitrile (94 : 3.5 : 2.5, by vol.) with 20 mM choline chloride (B) (Vogel and Eichenberger 1992). The gradient was from 70% to 100% B in 40 min with a flow rate of 1.5 ml min^{-1} and detection was at 202 nm. Molecular species of MGDG were separated in an isocratic manner with methanol/water (94 : 6, by vol.) as a solvent (Giroud et al. 1988). The flow rate was 1.1 ml min^{-1} and detection was at 210 nm. Single peaks, previously identified by GLC (Hofmann and Eichenberger 1997), were collected in vials and their radioactivity was directly measured by liquid scintillation counting after addition of 2 ml of methanol and 5 ml of 0.7% (w/v) butyl-PBD (Glaser) in toluene.

Results

In order to get some general information on the de novo synthesis of fatty acids and their distribution among particular lipids, *Dictyopteris membranacea* was incubated with [2-^{14}C]acetate in a pulse-chase manner. Lipids were extracted at different times during the chase and then separated by 2-dim. TLC. The radioactivity of particular lipids was quantified by liquid scintillation counting. For measuring the label in single fatty acids, their phenacylesters were prepared from an aliquot of each lipid sample and then separated by RP-HPLC. The incorporation of [2-^{14}C]acetate into lipids and fatty acids is shown in Fig. 1.

At the end of the 1 h pulse, PG was by far the most strongly labelled lipid (69%), followed by DGTA (17%) and SQDG (9.5%) (Fig. 1a). During the chase period of 6 d, the label decreased mainly in PG and, to a lesser extent, in DGTA, but increased in the glycolipids MGDG and SQDG. Almost no radioactivity was found in the phospholipids PHEG and PI and in the unpolar TAG (data not shown). Within fatty acids of polar lipids, at the end of the pulse, the bulk of radioactivity appeared in 18:1 (61%) and 16:0 (20%) indicating that these two fatty acids as the main products of de novo synthesis are immediately incorporated into lipids. During the chase the label decreased in 18:1, but increased in C_{18} and C_{20} polyunsaturated acids, predominantly in 20:4 (Fig. 1b).

Using [1-^{14}C]oleate as substrate, the total label found in polar lipids was around 50,000 dpm at the end of the 1 h pulse and 70,000 dpm at the end of the chase indicating this value to be almost constant during the experiment. At the end of the pulse, PG contained 39%, PE 23% and DGTA 22% of the total label of polar lipids, as shown in Fig. 2a.

During the chase, a decrease of radioactivity in these lipids and a concomitant increase in MGDG, SQDG and

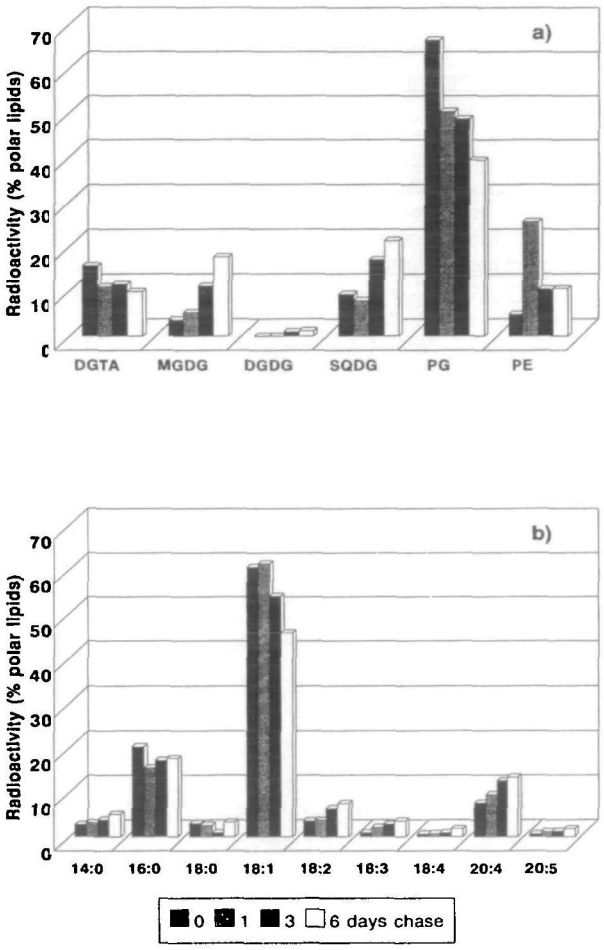

Fig. 1 Incorporation of [2-^{14}C]acetate into (a) polar lipids and (b) their fatty acids of *D. membranancea*. Conditions were as described in Materials and Methods.

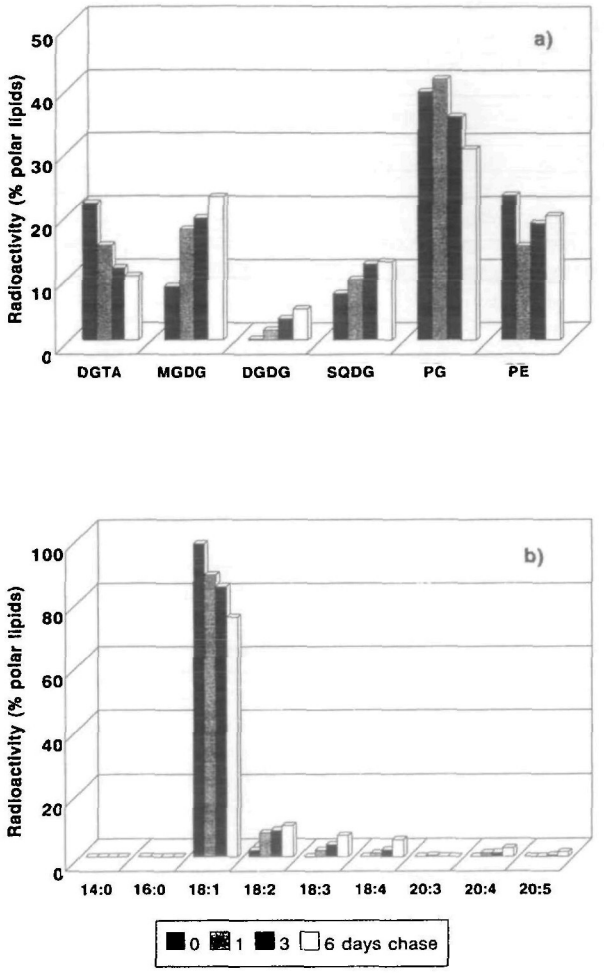

Fig. 2 Incorporation of [1-^{14}C]oleate into (a) polar lipids and (b) their fatty acids of *D. membranancea*. Conditions were as described in Materials and Methods.

DGDG was observed suggesting a transfer of label among polar lipids. Within fatty acids, 18:1 was the most strongly labelled acid which even at the end of the chase accounted for 75% of the total radioactivity in polar lipids (Fig. 2b). Only little label was shifted to polyunsaturated C_{18} acids, and C_{20} acids were not significantly labelled. It should be noted that only traces of radioactivity were found in 14:0 and 16:0 acids indicating that breakdown of [1-^{14}C]oleate and re-incorporation into fatty acids via de novo synthesis could almost be excluded. At the end of the pulse 90% of the total label from [1-^{14}C]oleate was found in the unpolar lipid fraction with TAG containing about one third of the radioactivity. This label rapidly disappeared from TAG without a concomitant increase in polar lipids suggesting that no significant exchange of label occurred between the polar and unpolar lipid fractions.

To get some insight into processes occurring on single lipids, the distribution of label among the fatty acids of particular lipids was determined. The data obtained for glycolipids are presented in Fig. 3.

At the end of the pulse, the label was mainly found in 18:1 in all the lipids examined. During the chase, an accumulation of the label in 18:2, 18:3 and 18:4 in the galactolipids and in 18:2 and 18:3 of SQDG was observed. After 6 d, C_{18} PUFAs accounted for 56% in MGDG and 65% in DGDG, but for 31% only in SQDG. Considering C_{20} acids, an accumulation of label in 20:5 of DGDG was observed.

The corresponding data of DGTA and the phospholipids PG and PE are shown in Fig. 4. In DGTA, the radioactivity in 18:1 rapidly decreased, but only minor parts of the label were found in 18:2 and 20:4 acids. Interestingly, within molecular species the rapid decrease of 18:1 exclusively occured in the 14:0/18:1 combination (not shown) suggesting a key role of this molecular species in the lipid metabolism of *D. membranacea*. In both PG and PE, the

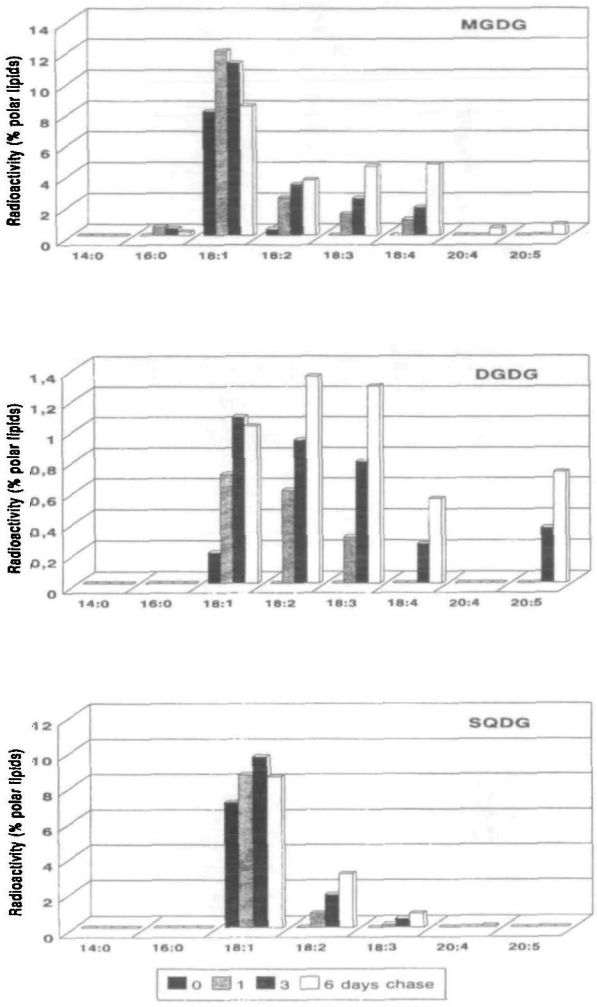

Fig. 3 Time course of radioactivity in fatty acids of MGDG, DGDG and SQDG from [1-^{14}C]oleate-labelled *D. membranacea*.

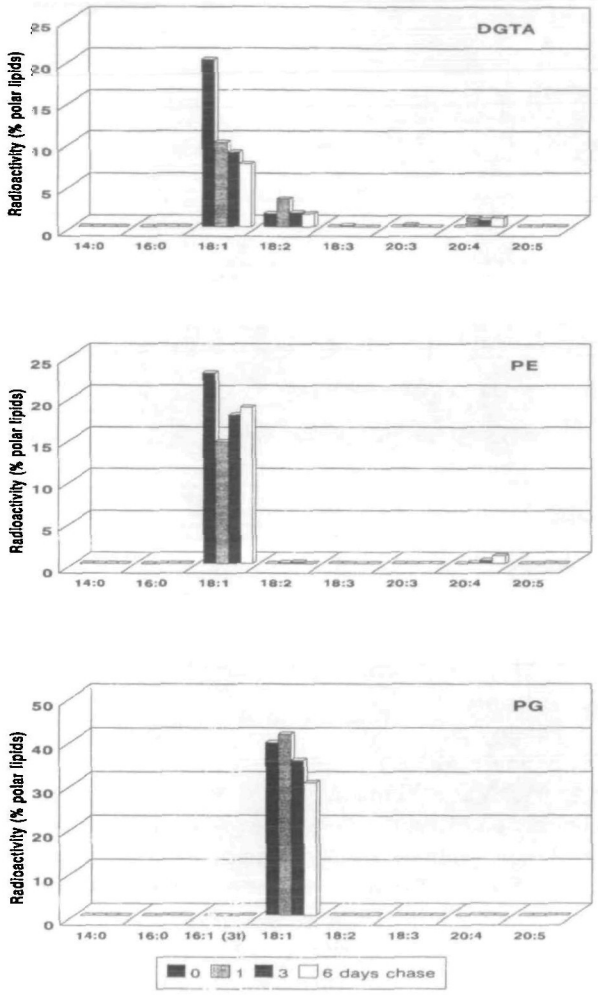

Fig. 4 Time course of radioactivity in fatty acids of DGTA, PG and PE from [1-^{14}C]oleate-labelled *D. membranacea*.

label appeared in 18:1 only. In PG, a decrease of label was observed, while in PE, the labelling kinetics are not clear. It should be kept in mind, however, that in different cellular compartments (plastids, ER, mitochondria) different pools of PG exist each of which might be involved differently in the cellular lipid metabolism. Thus, the role of both PG and PE remains to be more thoroughly investigated.

Since 14:0 is an important constituent fatty acid of DGTA (Hofmann and Eichenberger 1997) and since the 14:0/18:1 species of this lipid seemed to be rapidly metabolised in the above-mentioned experiment, *D. membranacea* was further incubated with [1-^{14}C] myristate. This substrate was rapidly incorporated into polar lipids but rather poorly metabolised, since, even at the end of the chase, 84% of the radioactivity in polar lipids was still found in 14:0 (not shown). Its incorporation into polar lipids is shown in Fig. 5. The label mainly appeared in DGTA (59%), PG (15%) and MGDG (9%). DGTA was the main acceptor of [1-^{14}C]14:0 which, during the chase, rapidly disappeared indicating again a rapid turnover of acyl groups in this betaine lipid. A slight decrease was also measured in PG. At the same time, the radioactivity accumulated mainly in the glycolipids and, to a minor extent, in the phospholipids PE and PHEG.

Since these results suggested a transfer of label from DGTA to MGDG, the labelling kinetics of the molecular species of these two lipids were measured. At the end of the pulse, the most strongly labelled molecular species of DGTA were 14:0/20:4, 14:0/18:2, 14:0/18:1 and 14:0/20:5 accounting for 80% of the total radioactivity in DGTA as demonstrated in Fig. 6. Interestingly, there was a decrease of label in all the 14:0-containing species during the chase period. In MGDG, the radioactivity mainly appeared and,

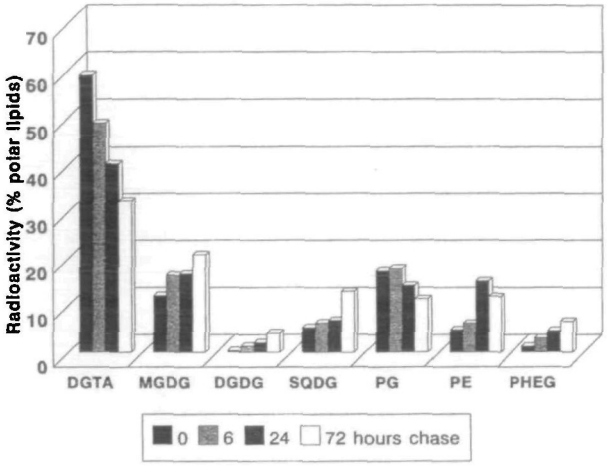

Fig. 5 Incorporation of [1-^{14}C]myristate into polar lipids of *D. membranacea*. Conditions were as described in Materials and Methods.

during the chase, even increased in 14:0/18:1 and 14:0/18:2 combinations suggesting a transfer of labelled 14:0 from DGTA to MGDG.

Finally, *D. membranacea* was incubated with [1-^{14}C]-arachidonate which is a precursor of 20:5 (n-3) in many algae (Arao and Yamada 1994, Khozin and Cohen 1996, Schneider and Roessler 1994). Much less, however, is known about the formation of this compound in brown algae.

DGTA and PE were the most strongly labelled components containing 51% and 26%, respectively, of the total label in polar lipids, as shown in Fig. 7. During the chase, the label in DGTA rapidly decreased to 27% indicating once again the high turnover of the acyl groups in the betaine lipid. At the same time, the label increased in the glycolipids and in PHEG, while the labelling kinetics of PG and PE were less clear.

It should be mentioned that 10% of the label in fatty acids esterified to polar lipids was found in 16:0, 18:1 and 18:2 acids (not shown) indicating the degradation of a small proportion of the substrate. The ratio of labelled 20:5 (n-3) to 20:4 (n-6) was approximately 1:3 in both galactolipids, 1:17 in SQDG and below 1:50 in DGTA and in the phospholipids (not shown) indicating the galactolipids to be the major candidates for the conversion of 20:4 (n-6) to 20:5 (n-3). Thus, molecular species of MGDG were analysed, as shown in Fig. 8.

The prominent finding is a clear decrease of label during the chase in the fraction containing 20:4 but not 20:5. The concomitant increase of label in the fraction containing 20:5 but not 20:4 strongly suggests a MGDG-linked desaturation of 20:4 (n-6) to 20:5 (n-3). Since in the other fractions, 20:4-containing species were not separated from 20:5-containing species, their labelling kinetics could not be interpreted.

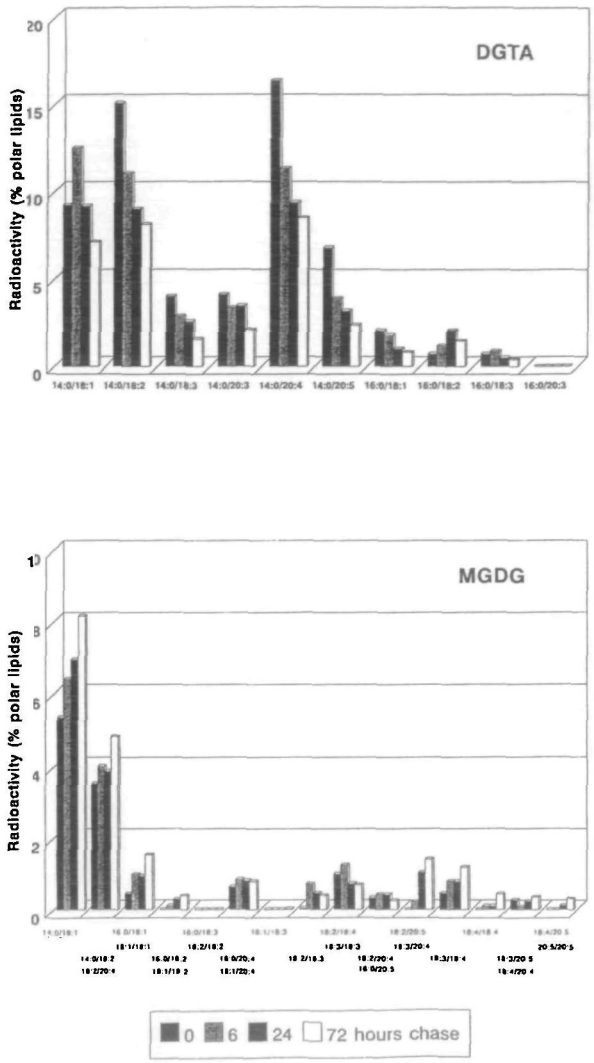

Fig. 6 Time course of label in molecular species of DGTA and MGDG from [1-^{14}C]myristate-labelled *D. membranacea*.

Discussion

There are few studies investigating the lipid metabolism of marine brown algae which produce long-chain PUFAs (Harwood and Jones 1989) and very often also the betaine lipid DGTA (Eichenberger et al. 1993), suggesting the biochemical pathways operating in these organisms to be different from those of higher plants. Our work focussed on *Dictyopteris membranacea* which, as a member of the Dictyotales, contains DGTA but no detectable amounts of PC (Hofmann and Eichenberger 1997). Pulse-chase experiments with [2-^{14}C]acetate and different [1-^{14}C] fatty acids were carried out in order to get some general insight into the lipid metabolism as well as into the role of DGTA in this alga.

The de novo synthesis of fatty acids in *D. mem-*

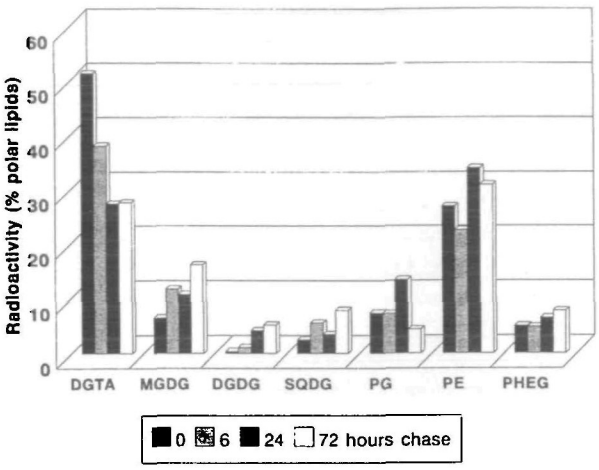

Fig. 7 Incorporation of [1-^{14}C]arachidonate into polar lipids of *D. membranacea*. Conditions were as described in Materials and Methods.

branacea was investigated using [2-^{14}C]acetate as a substrate. As in other brown algae (Harwood and Jones 1989) and in higher plants (Ohlrogge and Browse 1995), 16:0 and 18:1 were the main products. The further processing of these primary products, however, was rather slow in *D. membranacea*. The significant labelling of 20:4 acid from [2-^{14}C]acetate probably reflects the use of the applied substrate for elongation reactions (Cassagne et al. 1994).

Among polar lipids, PG was the main acceptor of de novo synthesised fatty acids, followed by the betaine lipid DGTA and by PE. PG and PE were also the main acceptors of [1-^{14}C]oleate as also observed in *Ectocarpus fasciculatus* (Makewicz et al. 1997) and PE was further significantly labelled from [1-^{14}C]arachidonate which is a major constituent fatty acid of this lipid. The labelling kinetics

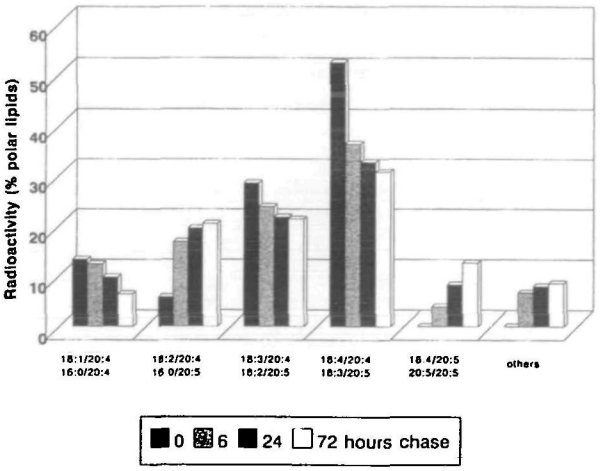

Fig. 8 Time course of radioactivity in molecular species of MGDG from [1-^{14}C]arachidonate-labelled *D. membranacea*.

of PE, however, were not unambigous in our experiments. Thus, for a final interpretation of the role of this lipid, additional investigations are necessary. In the plastidial glycolipids MGDG, DGDG and SQDG the label from all the substrates applied mainly accumulated during the chase periods.

DGTA was the main acceptor of exogenous [1-^{14}C]myristate and [1-^{14}C]arachidonate and also one of the main acceptors of [1-^{14}C]oleate. With all the substrates used DGTA showed a significant turnover of radioactivity. With [1-^{14}C]myristate, a decrease of radioactivity during the chase was found in all molecular species containing this fatty acid, whereas with [1-^{14}C]oleate, an intermediate labelling of mainly the 14:0/18:1 combination was observed. DGTA was also significantly labelled from [2-^{14}C]acetate due to the incorporation of de novo synthesised 16:0, 18:1 and 20:4 acids. This is in keeping with results from *Fucus serratus* (Smith and Harwood 1984), and *Ascophyllum nodosum* and *F. vesiculosus* (Jones and Harwood 1993) where DGTA was the most strongly labelled lipid after incubation with [1-^{14}C]acetate. Our results therefore point towards a key position of the betaine lipid in the incorporation of both preformed and de novo synthesised fatty acids and their redistribution among polar lipids.

An involvement of betaine lipids in the redistribution of fatty acids has also been suggested for DGTA in *Chroomonas salina* (Henderson and Mackinlay 1992), for DGTS in *Ochromonas danica* (Vogel and Eichenberger 1992) and for DGCC in *Pavlova lutheri* (Eichenberger and Gribi 1997). On the other hand, DGTA seems to play a different role in the unicellular algae *O. danica* (Vogel and Eichenberger 1992), *Cryptomonas* CR-1 (Sato 1991) and *P. lutheri* (Eichenberger and Gribi 1997, Kato et al. 1995), and in the brown alga *E. fasciculatus* (Makewicz et al. 1997) where it is strongly suggested to act as a final acceptor for polyunsaturated C_{20}- and C_{22} acyl chains.

It should be pointed out that after incubation with exogenous [1-^{14}C]fatty acids, TAG was strongly labelled. The label was weak, however, when [2-^{14}C]acetate was used as a substrate possibly indicating differences between the fluxes of endogenous and exogenously supplied fatty acids. In all experiments, however, a rapid decrease of radioactivity in TAG but no corresponding increase in the polar lipids was observed. The physiological importance of this process needs to be cleared.

Considering desaturation processes, the incorporation of label from [1-^{14}C]oleate into the fatty acids of the glycolipids provided evidence for a 18:1 to 18:3 desaturation in SQDG and a 18:1 to 18:4 desaturation in the galactolipids. Especially the labelling kinetics of the different C_{18}/C_{18} species of MGDG were in favour of a lipid-linked process which is well known from higher plants (Schmidt and Heinz 1993) and algae (Giroud and Eichenberger 1989, Makewicz et al. 1997, Stern and Tietz 1993). Furthermore

the galactolipids appeared to be the only site of 18:4 acid production in *D. membranacea* as also observed in *E. fasciculatus* (Makewicz et al. 1997), *Isochrysis galbana* (Stern and Tietz 1993) and *Echium plantagineum* (Williams and Khan 1996).

In contrast, in PG and PE no further processing of fatty acids was observed under our experimental conditions, although these phospholipids were strongly labelled from [1-^{14}C]oleate. In DGTA, a slight labelling of 18:2 from [1-^{14}C]oleate might indicate DGTA to be involved in desaturation processes. A lipid-linked desaturation occurring on betaine lipids has already been suggested for the conversion of 22:5 to 22:6 in DGTA of *P. lutheri* (Eichenberger and Gribi 1997), of 18:1 to 18:2 in DGTS of *O. danica* (Vogel and Eichenberger 1992) and of 18:1 to 18:4 acids in DGTS of *Chlamydomonas reinhardtii* (Giroud and Eichenberger 1989).

The incubation of *D. membranacea* with [1-^{14}C]arachidonate which was shown to be a precursor for 20:5 (n-3) acid in several algae (Arao and Yamada 1994, Khozin and Cohen 1996, Schneider and Roessler 1994) shed some light on the 20:4 (n-6) to 20:5 (n-3) conversion in this brown alga. The labelling kinetics of the molecular species of MGDG strongly suggest this lipid to act as a substrate. The results even suggest that the galactolipids are the exclusive site of 20:5 (n-3) production under our experimental conditions. This is in full agreement with data from the red alga *Porphyridium cruentum* where a MGDG-linked desaturation of 20:4 (n-6) to 20:5 (n-3) was observed, too (Khozin et al. 1997). In contrast, the 20:5 (n-3) acid of extraplastidial lipids in the same organism is thought to be synthesised by a $\Delta 5$ desaturation of 20:4 (n-3) acid (Shiran et al. 1996). This may also be the case in *D. membranacea*, since no significant conversion of [1-^{14}C]20:4 (n-6) into 20:5 (n-3) acid has been found in extraplastidial lipids of this alga. On the other hand, studies with *Phaeodactylum tricornutum* (Arao and Yamada 1994) and a 20:5 (n-3)-deficient mutant of *Nannochloropsis* sp. (Schneider et al. 1995) clearly demonstrated the desaturation of 20:4 (n-6) to 20:5 (n-3) acid to occur exclusively in the cytoplasm with a subsequent export of 20:5 (n-3) to the chloroplast. A similar pathway is also suggested for *P. lutheri* (Eichenberger and Gribi 1997). This indicates that for the synthesis of 20:5 (n-3) acid, different pathways at various sites may operate in different organisms as pointed out in detail for *P. cruentum* and *Monodus subterraneus* (Khozin and Cohen 1996).

An important characteristic of *D. membranacea* is the absence of detectable amounts of PC which plays a major role in the general concept of glycerolipid biosynthesis (Roughan and Slack 1982). In higher plants, this phospholipid is generally accepted to act as a donor of cytoplasmic DAG precursors used for the synthesis of eukaryotic plastidial lipids characterised by containing C_{18} acyl chains in their *sn*-2 position (Frentzen 1986). According to this concept, the absence of PC should result in the presence of glycolipids of the prokaryotic type only, as shown for the green alga *C. reinhardtii* (Giroud et al. 1988). In *D. membranacea*, however, the galactolipids are almost exclusively of the eukaryotic type (Hofmann and Eichenberger 1997) as found also in several members of the Fucales (Jones and Harwood 1992) which all lack PC.

This strongly suggests an alternative pathway to be responsable for the biosynthesis of eukaryotic galactolipids in these organisms. One possibility is the production of eukaryotic DAG in the plastid itself by the action of acyltransferases with substrate specificities for C_{18}-residues. This idea has already been put forward for the unicellular algae *Cryptomonas* CR-1 (Sato 1991) and *P. lutheri* (Eichenberger and Gribi 1997, Kato et al. 1995) which showed a rapid labelling of eukaryotic galactolipids without an intermediate labelling of any other precursors. Direct evidence for such a pathway on the level of enzymes, however, is missing as yet. In *D. membranacea*, in contrast, the galactolipids were rather slowly labelled with all the substrates used possibly indicating that the galactolipids are formed from lipids other than PC as donors of the eukaryotic DAG-moieties. Since in DGTA a high turnover of label was observed with all the substrates used, it is tempting to assume the betaine lipid to be involved in this pathway. This is also supported by the decrease of labelled 14:0/18:1 and 14:0/18:2 molecular species in DGTA and the simultaneous increase of label in the same molecular species in MGDG. From these precursors, the other molecular species of MGDG could easily be formed by transacylation. A rapid transacylation could be demonstrated in our experiments by the incorporation of [1-^{14}C]arachidonate which is located exclusively in the *sn*-1 position of MGDG in *D. membranacea* (Hofmann and Eichenberger 1997) as well as in other brown algae (Arao and Yamada 1989, Jones and Harwood 1992).

Although additional experiments will have to definitely clear up the role of DGTA in lipid biosynthesis, our data obtained from *D. membranacea* generally point towards alternative biosynthetic pathways differing from those of higher plants.

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