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Betaine Lipids

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Diacylglyceryltrimethylhomoserine (DGTS) and diacylglyceryl hydroxymethyltrimethyl-β-alanine (DGTA) belong to a new type of glycerolipids called betaine lipids, which are composed of diacylglycerol and *N*-permethylated hydroxyamino acids, that are linked by an ether linkage. Betaine lipids are widely distributed in lower plants and algae as well as in some non-photosynthetic microorganisms. They are no longer regarded as "unusual" lipids but are important constituents of membranes of lower organisms. The present state of knowledge of the phylogenetic distribution, the fatty acid composition, the thermal properties, and the biosynthesis of the bataine lipids is briefly summarized in this review in a perspective of a future search for the biological roles and activities of betaine lipids.

Key words: Algal lipid — Betaine lipid — DGTA — DGTS — Fatty acid — Methionine

The betaine lipids constitute a new group of complex lipids, which used to be classified into phospholipids and glycolipids before the discovery of DGTS. Betaine lipids are characterized by their peculiar structure: a permethylated derivative of hydroxyamino acid is linked to a diacylglycerol through an ether bond. At least two types of betaine lipids are known to occur in various species of lower plants and algae. The presence of these betaine lipids in a chrysophyte *Ochromonas danica* was first noted by Nichols and Appleby (1969) and Nichols (1970). The chemical structure of DGTS was established by Brown and Elovson (1974), whereas the complete elucidation of the structure of DGTA was achieved quite recently (Vogel *et al.*, 1990, see also Sato 1991a). Interestingly, the polar parts of DGTS and DGTA are structural isomers (Fig. 1). Both have a positively charged trimethyl ammonium group and a negatively charged carboxyl group, and thus they are zwitterionic at neutral pH. Although the betaine lipids have been considered as "unusual lipids", this misunderstanding can no longer be justified in the light of the present state of knowledge of the betaine lipids. The two types of betaine lipids are widely distributed among various species of lower plants and algae and, in many species, either DGTS or DGTA is a major lipid component.

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Abbreviations: DGTA = 1(3), 2-diacylglyceryl-3(1)-*O*-2′-(hydroxymethyl)(*N*, *N*, *N*trimethyl)-β-alanine; DGTS = 1(3), 2-diacylglyceryl-3(1)-*O*-4′-(*N*, *N*, *N*-trimethyl)homoserine; MGDG = monogalactosyl diacylglycerol; PC = phosphatidylcholine.

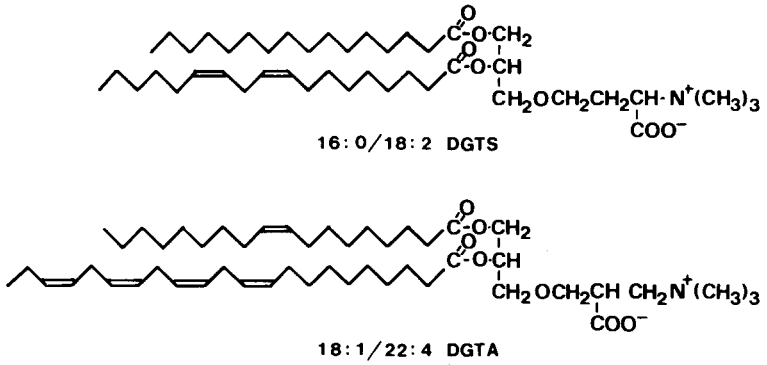

Fig. 1. Structure of DGTS and DGTA. A representative molecular species of DGTS in *Adiantum* (Sato and Furuya 1983) and a representative molecular species of DGTA in *Cryptomonas* (Sato 1991a) are shown as examples.

This review will present a comprehensive summary of recent developments in the study of the structure, distribution and biosynthesis of betaine lipids. It is relevant to consult a recent review on the algal lipids (Harwood and Jones 1989).

I. Chemical Identification of Betaine Lipids

Betaine lipids can be extracted from algal cells or plant materials by conventional techniques of lipid analysis (Bligh and Dyer 1959, Kates 1972). They are conveniently detected and analyzed by two-dimensional thin-layer chromatography. The solvent systems developed by Nichols and Appleby (1969), Sato and Furuya (1983), Sato *et al.* (1988), and Vogel *et al.* (1990) are all useful in completely separating the betaine lipids from one another and from other types of lipids. Nevertheless, the use of acidic solvents in the development is not recommended, since DGTA is now known to be readily deaminated in acidic solutions under certain conditions that are not yet clearly defined (Vogel *et al.*, 1990). A typical separation of bataine lipids by two-dimensional thin-layer chromatography is shown in Sato (1991a). Dragendorff reagent (Kates 1972) is used to stain betaine lipids and phosphatidylcholine on the chromatogram, although this reagent also reacts weakly with galactolipids. Betaine lipids are not stained with a phospholipid-staining reagent (Dittmer-Lester reagent, see Kates 1972).

The conclusive identification of betaine lipids can be achieved by NMR spectroscopy. Both ^{1}H-NMR (for DGTS : Brown and Elovson 1974, Evans *et al.*, 1982a, Sato and Furuya 1983 ; for DGTA : Vogel *et al.*, 1990) and ^{13}C-NMR (for DGTS : Evans *et al.*, 1982a ; for DGTA : Vogel *et al.*, 1990, Sato 1991a) are useful in establishing the structure of these lipids. The signal due to trimethylamino group appears at about 3.3 ppm in ^{1}H-NMR and at 54 ppm in ^{13}C-NMR. The signals for the C_4 backbone are clearly separated from the signals due to glycerol and fatty acyl groups and thus are useful in identifying the betaine lipids.

Infrared absorption spectroscopy (for DGTS: Eichenberger and Boschetti 1978, Sato and Furuya 1983; for DGTA: Vogel *et al.*, 1990, Araki *et al.*, 1991) and mass spectrometry (for DGTS: Eichenberger and Boschetti 1978, Sato and Furuya 1983; for DGTA: Vogel *et al.*, 1990, Sato 1991a) are also useful in confirming the structure of betaine lipids.

II. Phylogenetic Distribution of Betaine Lipids

Following its discovery in a chrysophyte (Brown and Elovson 1974), DGTS has been found in various species of green algae including *Chlamydomonas reinhardtii* (Eichenberger and Boschetti 1978), *Volvox carteri* (Moseley and Thompson 1980) and several species of *Dunaliella* (Evans *et al.*, 1982a, 1982b, Fried *et al.*, 1982). In 1983, we reported the presence of DGTS in a fern *Adiantum capillus-veneris* (Sato and Furuya 1983). This finding was especially unexpected because this was the first report of the detection of DGTS in a vascular plant. DGTS has been detected in most species of pteridophytes (Sato and Furuya 1984a), bryophytes (Sato and Furuya 1985) and green algae (Eichenberger 1982, Sato and Furuya 1985). No species of seed plants (both angiosperms and gymnosperms) are known to contain DGTS until now. Therefore, DGTS is widely distributed among cryptogamic green plants (Table 1). DGTS is also found in several species of algae that contain chlorophylls *a* and *c* (Brown and Elovson 1974, Sato *et al.*, 1988, Sato 1991a), as well as in some non-photosynthetic organisms such as fungi (Yamada and Nozawa 1979, Vaskovsky *et al.* 1991) and a protozoon (Furlong *et al.*, 1986). It is interesting to note that the content of DGTS and that of PC seem to be reciprocal to each other (Eichenberger 1982, Sato and Furuya 1985). The organisms that accumulate high levels of DGTS do not contain PC (eg., *Chlamydomonas reinhardtii*) or contain very low levels of PC (eg., *Ulva pertusa*, *Chaetomorpha spiralis*). This reciprocity between DGTS and PC can be interpreted to indicate that DGTS has been progressively replaced by PC during the process of evolution of green plants.

The occurrence of DGTA is mostly restricted to the algae that contain chlorophylls *a* and *c*. Fucales and Dictyotales are representative groups of brown algae that accumulate high levels of DGTA (Araki *et al.*, 1991). Interestingly, these algae do not contain detectable amounts of PC. Apparently no DGTS is detected in those algae that contain DGTA. Nevertheless, if DGTA is synthesized uniquely from DGTS as demonstrated in *Ochromonas danica* (Vogel and Eichenberger 1991) and *Cryptomonas* sp. (Sato 1991b), all of the species of algae that contain DGTA are supposed to contain low levels of DGTS as a precursor to DGTA (*vide infra*).

III. Intracellular Localization of Betaine Lipids

Betaine lipids are components of biological membranes as are other classes of complex lipids. DGTS is present in both haploid (gametophyte) and diploid (sporophyte) tissues of a fern *Adiantum capillus-veneris* (Sato and Furuya 1984b). In the

Table 1. Distribution of betaine lipids in plants, algae and microorganisms

Division Species	Content (nmol/mg or % total lipid)		Reference
	DGTS	DGTA	
Angiosperms	–	–	a
Gymnosperms	–	–	a
Pteridophytes			
Psilotum nudum	–	–	a
Lycopodium clavatum	52	–	a
Selaginella uncinata	54	–	a
Equisetum arvense	18	–	a
Equisetum hiemale	8	–	a
Ophioglossum thermale	1	–	a
Botrychium virginianum	2	–	a
Angiopteris lygodiifolia	3	–	a
Osmunda japonica	21	–	a
Lygodium japonicum	30	–	a
Adiantum capillus-veneris	30	–	a
Pteris vittata	68	–	a
Dryopteris crassirhizoma	8	–	a
Polypodium formosanum	4	–	a
Asplenium unilaterale	14	–	a
Salvinia cucullata	57	–	a
Bryophytes			
Pleurozium schreberi	14	–	b
Dicranum scoparium	7	–	b
Polytrichum formosum	13	–	b
Bazzania yoshinagana	36	–	b
Marchantia paleacea	15	–	b
Marchantia polymorpha	(1.8 %)	–	c
Chlorophytes (green algae)			
Chara australis	6	–	b
Nitella axilliformis	28	–	b
Coleochete scutata	78	–	b
Closterium acerosum	96	–	b
Chlorella vulgaris	–	–	b
Chlorella pyrenoidosa	–	–	d
Chlorella fusca	(1.3 %)	–	e
Chlamydomonas reinhardtii C-238	114	–	b
Chlamydomonas reinhardtii 137c	120	–	d
Dunaliella parva	(14.2 %)	–	f
Dunaliella tertiolecta	(7.5 %)	–	f
Dunaliella bardawil	(13.4 %)	–	g
Volvox carteri	+	–	h
Codium mamillosum	6	–	b
Codium fragile	83	–	b
Caulerpa brachypus	27	–	b
Acetabularia calyculus	158	–	b
Chaetomorpha spiralis	60	–	b
Cladophora japonica	102	–	b

Table 1. (continued)

Division Species	Content (nmol/mg or % total lipid)		Reference
	DGTS	DGTA	
Monostroma sp.	118	–	b
Ulva pertusa	160	–	b
Polytoma uvella	63	?	d
Fritschiella tuberosa	5	?	d
Prototheca zopfii	–	?	d
Prasinophytes			
Pyramimonas parkeae	–	–	b
Tetraselmis convolutae	–	–	b
Euglenophytes			
Euglena gracilis	–	?	d
Phaeophytes (brown algae)			
Fucus vesiculosus	–	+	d, i
Fucus serratus	–	(7.5 %)	j
Ascophyllum nodosum	–	+	i
Hizikia fusiformis	–	(15.2 %)	k
Sargassum horneri	–	(14.1 %)	k
Sargassum ringgoldianum	–	(12.6 %)	k
Sargassum thunbergii	–	(20.5 %)	k
Dictyota dichotoma	–	(19.7 %)	k
Pachydictyon coriaceum	–	(14.8 %)	k
Padina arborescens	–	(10.9 %)	k
Ishige okamurai	–	(5.6 %)	k
Colpomenia sinuosa	–	–	k
Endarachne binghamiae	–	–	k
Scytosiphon lomentarius	–	–	k
Undaria pinnatifida	–	–	k
Eisenia bicyclis	–	–	k
Raphidophytes			
Chattonella antiqua	(5.8 %)	–	l
Chrysophytes			
Ochromonas danica	+	(5.0 %)	i, m
Cryptophytes			
Cryptomonas sp. CR-1	(1.0 %)	(9.7 %)	n
Fungi			
Epidermophyton floccosum	(15.0 %)	?	o
Boletus edulis	+	?	p
Protozoa			
Acanthamoeba castellanii	+	?	q

+, present but no description of the content; –, undetectable; ?, no description.
References: a, Sato and Furuya 1984a; b, Sato and Furuya 1985; c, Sato and Kato, 1988; d, Moseley and Eichenberger 1982; e, Weber *et al.*, 1989; f, Evans *et al.* 1982b; g, Fried *et al.*, 1982; h, Thompson 1980; i, Vogel *et al.*, 1990; j, Smith and Harwood 1984; k, Araki *et al.*, 1991; l, Sato *et al.*, 1988; m, Brown and Elovson 1974; n, Sato 1991a; o, Yamada and Nozawa 1979; p, Vaskovsky *et al.*, 1991; q, Furlong *et al.*, 1986.

sporophyte, DGTS is present at comparable levels in both stipe and pinna, although the latter is highly differentiated as a principal photosynthetic organ.

There are reports on the presence of DGTS in the chloroplasts of *Chlamydomonas*. Both thylakoid membranes (Janero and Barrnett 1982) and envelope membranes (Mendiola-Morgenthaler *et al.*, 1985) have been shown to contain DGTS. These findings do not necessarily mean that the chloroplast is the major site where DGTS accumulates. In fact, isolated plasma membranes of *Dunaliella salina* (Sheffer *et al.*, 1986) contained a high concentration of DGTS. The extremely high concentration of DGTS in the cell of *Chlamydomonas* (Giroud *et al.*, 1988) can only be explained if DGTS is the major lipid component in the membranes outside the chloroplast. No experimental results have been obtained on the intracellular localization of DGTA.

IV. Fatty Acid Composition of Betaine Lipids

DGTS has been isolated from various species of plants and algae, and its fatty acid composition has been analyzed in detail (Table 2). The analysis of fatty acids esterified to the C-1 and C-2 of glycerol shows an interesting feature of this lipid class. Major fatty acids at the C-1 are saturated acids of C_{16} (palmitic) in most of the organisms analyzed and C_{14} (myristic) in *Ochromonas*. The fatty acids at the C-2 are unsaturated acids of C_{18} in most organisms analyzed to date and C_{20} in *Chattonella antiqua*. This type of positional distribution of fatty acids at the two carbon atoms of glycerol of DGTS has been interpreted to represent that the diacylglycerol moiety of DGTS is synthesized by the action of the acyltransferases in the endoplasmic reticulum. This inference is based on the analogy to the two-pathway hypothesis on the synthesis of molecular species of lipids in higher plants (Roughan and Slack 1982, Somerville and Browse 1991). According to this hypothesis, the diacylglycerol moiety of the "prokaryotic" lipids, which are difined as lipids that contain C_{16} acids at the C-2 of glycerol, are synthesized in the plastid by the action of glycerol-3-phosphate acyltransferase in the stroma and lysophosphatidate acyltransferase in the envelope (plastidial pathway). The diacylglycerol moiety of the "eukaryotic" lipids, which contain C_{18} (unsaturated) acids at the C-2 of glycerol and, most often, saturated acids at the C-1 of glycerol, are synthesized by the action of the two acyltransferases located in the endoplasmic reticulum (cytoplasmic pathway). This hypothesis is proposed for the lipid biosynthesis in higher plants, but it is still unknown whether this is true for the lipid biosynthesis in algae in general, since the galactolipids in *Cryptomonas* that contain C_{18} acids at both C-1 and C-2 do not seem to be synthesized by the cytoplasmic pathway (Sato 1991b).

The fatty acid composition of DGTA (Table 3) has been published in several organisms, and shows a distinct difference from the fatty acid composition of DGTS. The fatty acids of DGTA are generally highly unsaturated: C_{20} and C_{22} acids with 4 or 5 double bonds are the major components of DGTA. In both *Cryptomonas* and *Ochromonas*, in which DGTS and DGTA are present, the fatty acid composition of the two betaine lipids were totally different, and this difference could not be accounted for

Table 2. Fatty acid composition of DGTS from various species of plants and algae

Fatty acid	*Chlamydomonas reinhardtii*		*Chlorella fusca*		*Marchantia polymorpha*		*Adiantum capillus-veneris*		*Chattonella antiqua*		*Ochromonas danica*	
	C-1	C-2	C-1	C-2	C-1	C-2	C-1	C-2	C-1	C-2	C-1	C-2
14 : 0	0	0	—	—	—	—	—	—	1	0	41	3
16 : 0	58	3	46	5	44	2	44	0	48	1	4	—
16 : 1	2	7	5	16	2	2	—	—	0	0	—	—
Other C16	0	4	1	8	0	0	0	0	0	0	0	0
18 : 0	2	0	1	1	0	0	1	0	0	0	1	0
18 : 1	7	2	36	37	0	21	1	4	0	0	1	12
18 : 2	17	11	5	13	0	11	0	20	0	0	1	16
18 : 3 (9, 12, 15)	7	3	3	12	1	6	0	21	0	0	0	1
18 : 3 isomers	5	59	—	—	—	—	0	1	0	0	0	4
18 : 4	1	9	0	0	0	1	—	—	0	0	—	—
20 : 3	0	0	—	—	—	—	—	—	0	0	0	8
20 : 4	0	0	—	—	1	1	1	3	1	10	1	2
20 : 5	0	0	—	—	0	0	—	—	0	35	—	—
Other C20	0	0	—	—	—	—	0	0	0	0	0	0
C22	0	0	—	—	—	—	0	0	0	0	0	2
Others	0	0	3	8	2	6	3	1	0	0	1	2
Total	100	100	100	100	50	50	50	50	50	50	50	50

Compositions of fatty acids at the C-1 and C-2 glycerol in DGTS are shown. The total is 100 or 50 depending on the source of data.
References: *Chlamydomonas*, Giroud *et al.* (1988); *Chlorella*, Weber *et al.* (1989); *Marchantia*, Sato and Kato (1988); *Adiantum*, Sato and Furuya (1983); *Chattonella*, Sato *et al.* (1988); *Ochromonas*, Sato (unpublished results).

simply by the difference in extent of desaturation, which occurs on intact complex lipids in plants (for reviews, see Roughan and Slack 1982, Somerville and Browse 1991). To explain the difference in fatty acid compositions of DGTS and DGTA, Vogel and Eichenberger (1991) proposed that, during or after the transformation of DGTS to DGTA, the acyl groups are extensively exchanged. This idea was supported by the fact that the acyl groups of DGTA are subject to rapid turnover in *Fucus* (Smith and Harwood 1984) and in *Cryptomonas* (Sato 1991b).

V. Physico-chemical Properties of Betaine Lipids

The apparent reciprocity of the abundance of DGTS and PC among different species of plants and algae as described above (section II) suggests that DGTS and PC are similar in their chemical and physical properties and approximately interchangeable with each other in their roles within the cell. From this point of view, it is interesting to compare the physico-chemical properties of DGTS and PC. We measured, by differential scanning calorimetry and fluorescence depolarization, the temperature for the transition from the gel state to the liquid-crystalline state (T_m) of the aqueous dispersions of DGTS (Sato and Murata 1991). The results with 1-palmitoyl-2-stearoyl DGTS and 1, 2-distearoyl DGTS showed that both DGTS and PC are subject to a similar thermal phase transition except that the T_m of DGTS was 6°C higher than

Table 3. Fatty acid composition of DGTA from various species of algae

Fatty acid	*Ochromonas danica*	*Cryptomonas* CR-1		*Hizikia fusiformis*
		C-1	C-2	
14 : 0	1	0	0	6
16 : 0	3	9	0	26
16 : 1	0	1	0	2
18 : 0	15	5	1	0
18 : 1	5	12	1	3
18 : 2	4	0	0	7
18 : 3	0	5	1	3
20 : 2	6	0	0	1
20 : 3	4	0	0	0
20 : 4	13	0	2	37
20 : 5	0	13	9	8
22 : 4	3	2	26	0
22 : 5	43	0	1	0
22 : 6	0	0	6	0
Others	4	2	3	6
Total	100	50	50	100

References: *Ochromonas*, Vogel *et al.* (1990); *Cryptomonas*, Sato (1991a); *Hizikia*, Araki *et al.* (1991).

that of PC. Nothing is known about the thermal properties of DGTA, which is unstable when purified.

VI. Biosynthesis of Betaine Lipids

A. Origin of the polar group of DGTS

One of the most important question about the biosynthesis of the betaine lipids concerns the origin of the polar group and the mechanism of formation of the ether linkage between the glycerol and the hydroxyamino acids. Although homoserine was supposed to be a candidate for a precursor to the polar group of DGTS, exogenously added homoserine did not interfere with the photosynthetic labelling of the polar group of DGTS in *Chlamydomonas* (Sato 1988). L-Methionine was, however, effective in lowering the incorporation of photosynthetically fixed $^{14}CO_2$ into the polar group of DGTS. This result was taken to indicate that methionine is a precursor to the polar group of DGTS (Fig. 2). Radioactively labelled L-methionine was, in fact, incorporated specifically into the polar group of DGTS. Interestingly, the C-1 (carboxyl carbon), the C-3 plus C-4, and the S-methyl carbon of methionine were all incorporated into the polar group of DGTS (Sato 1988). These findings suggest that methionine plays a dual role in the biosynthesis of DGTS: the C_4 backbone of methionine is a precursor to the C_4 backbone of the polar group of DGTS, while the S-methyl group of methionine is a precursor to the N-methyl groups of DGTS. It is still unknown how many molecules of methionine are necessary to make one molecule of DGTS, since it is possible to make a DGTS molecule from three molecules of methionine if the first methyl group of methionine moves to the amino group by intramolecular migration. It is also unknown whether methionine is the sole donor of the three methyl groups. The role of methionine in the biosynthesis of DGTS has been confirmed in cultured cells of a liverwort *Marchantia polymorpha* (Sato and Kato 1988), in *Ochromonas danica* (Vogel and Eichenberger 1991) and in *Cryptomonas* sp. (Sato 1991b). In spite of continuing efforts, the mechanism of formation of the ether linkage of DGTS is still unknown. The crucial step toward the elucidation of this mechanism will be the establishment of an *in*

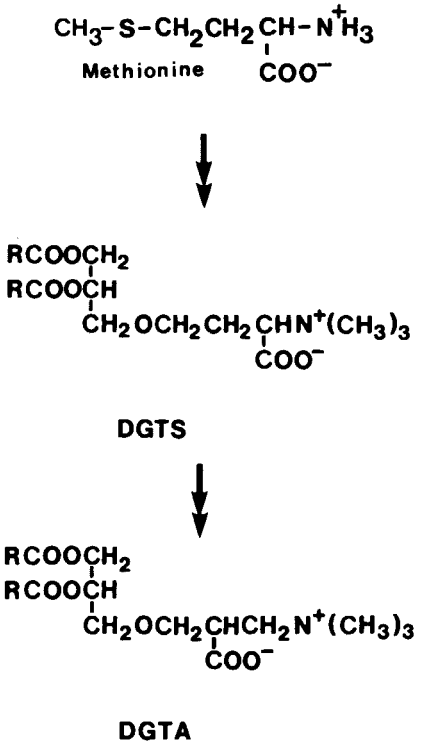

Fig. 2. A proposed pathway of the synthesis of betaine lipids. Each step is assumed to be composed of several different reactions.

vitro system of synthesis of DGTS.

B. Transformation of DGTS to DGTA

DGTA is not significantly labelled by in vivo labelling experiments (Sato 1991b) and this can be accounted for by the synthesis of DGTA from other classes of lipids. A reasonable candidate for the precursor to DGTA was its isomer DGTS. An evidence for the precursor-product relation of DGTS to DGTA was obtained by a pulse-labelling of *Ochromonas* cells with [3, 4-^{14}C]methionine and a chase for over 50 hr (Vogel and Eichenberger 1991). A direct proof for the conversion of DGTS to DGTA (Fig. 2) was obtained in an experiment in which doubly labelled DGTS (^{3}H in glycerol part and ^{14}C in polar part) was exogenously added to *Ochromonas* cells (Vogel and Eichenberger 1991). A clear precursor-product relationship between DGTS and DGTA was also demonstrated in *Cryptomonas* sp. during a chase period of 10 hr after a pulse labelling with either [*methyl*-^{14}C]methionine or [3, 4-^{14}C]methionine (Sato 1991b).

The mechanism of the transformation of DGTS to DGTA is still obscure. Since the C-1 (carboxyl carbon) is not conserved during the transformation, a possibility of migration of the carboxyl group to the vicinal carbon is excluded. The carboxyl group must be removed from DGTS and then another carboxyl group should be introduced to the vicinal carbon atom. The donor of the latter carboxyl group remains to be identified.

C. Modification of acyl groups

The acyl groups of DGTS are rapidly labelled by exogenously added fatty acids. Schlapfer and Eichenberger (1983) showed that the labelled oleic acid which was incorporated into DGTS was desaturated to linoleic acid (C_{18} acid with two double bonds at C-9, 10 and C-12, 13) and to an isomer of linolenic acid (C_{18} acid with three double bonds at C-5, 6, C-9, 10, and C-12, 13) in *Chlamydomonas*. The substrate for the desaturation was shown to be DGTS itself. The lipid-linked desaturation of acyl groups has been shown in PC (Stymne and Appelqvist 1978) and MGDG (Siebertz and Heinz 1977, Schmidt and Heinz 1990) of higher plants, in PC of a green alga *Chlorella* (Gurr 1971), and in MGDG of cyanobacteria (Appleby *et al.*, 1971, Sato *et al.*, 1986). It is quite reasonable that an organism like *Chlamydomonas* that lacks PC utilizes DGTS as a substrate for the desaturation of fatty acids.

The fatty acid composition of DGTA seems to be modified by acyl exchange. A comparison of the fatty acid compositions of DGTS and DGTA suggests that the fatty acyl groups must be replaced during or after the transformation of DGTS to DGTA (Vogel and Eichenberger 1991). The finding that photosynthetically assimilated carbon is more rapidly incorporated into the acyl groups of DGTA than into the polar group of DGTA gives a support for this hypothesis (Sato 1991b). Fatty acids at both C-1 and C-2 of glycerol moiety are apparently exchanged, but the enzymes involved in this acyl exchange are not investigated to date.

Prospects

Betaine lipids draw special attention of lipid biochemists, since they present more or less clear phylogenetic distributions in the plant kingdom, in contrast with other lipids such as phospholipids and glycolipids that are almost ubiquitous in plants. The investigation on the phylogenetic distribution of betaine lipids, which is still in progress, has suggested that DGTS is present in cryptogamic green plants and in many species of green algae, as well as in certain other species of algae that contain chlorophylls *a* and *c*, whereas the distribution of DGTA is rather limited within certain species of algae that contain chlorophylls *a* and *c*. Nevertheless, the results of biosynthetic studies of bataine lipids have revealed that DGTA is synthesized from DGTS. This finding necessitates further search for small amount of DGTS in those organisms that apparently contain DGTA but not DGTS. This interplay between biochemical studies and phylogenetic searches will be useful in elucidating the biological roles of betaine lipids in lower organisms. The only role of DGTS known to date is that DGTS acts as a carrier of acyl groups in fatty acid desaturation. Investigations on the precise location of the betaine lipids within the cell will provide a further clue for the research of the roles of these lipids.

Although most of the major complex lipids in green plants, green algae and brown algae are now identified, there are still a number of chemically unidentified lipids in lower plants and algae, e.g., Lipid U in *Marchantia* (Sato *et al.*, 1988) and Lipid X in *Cryptomonas* (Sato 1991a). A third type of betaine lipid may be among them. It is also quite possible that an intermediate of the conversion of DGTS to DGTA is found among them. We will have to continue our efforts to identify unknown lipids within the conceptual framework of current biochemistry and metabolism of lipids that include betaine lipids.

References

Appleby, R.S., R. Safford and B.W. Nichols. 1971. The involvement of lecithin and monogalactosyl diglyceride in linoleate synthesis by green and blue-green algae. Biochim. Biophys. Acta **248**: 205-211.

Araki, S., W. Eichenberger, T. Sakurai and N. Sato. 1991. Distribution of diacylglyceryl-hydroxymethyltrimethyl-β-alanine (DGTA) and phosphatidylcholine in brown algae. Plant Cell Physiol. **32**: 623-628.

Bligh, E.G. and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **37**: 911-917.

Brown, A.E. and J. Elovson. 1974. Isolation and characterization of a novel lipid, 1(3), 2-diacylglyceryl-(3)-*O*-4′-(*N*, *N*, *N*-trimethyl)homoserine, from *Ochromonas danica*. Biochemistry **13**: 3476-3482.

Eichenberger, W. 1982. Distribution of diacylglyceryl-*O*-4′-(*N*, *N*, *N*-trimethyl)homoserine in different algae. Plant Sci. Lett. **24**: 91-95.

——— and A. Boschetti. 1978. Occurrence of 1(3), 2-diacylglyceryl-(3)-*O*-4′-(*N*, *N*, *N*-trimethyl)-homoserine in *Chlamydomonas reinhardi*. FEBS Lett. **88**: 201-204.

Evans, R.W., M. Kates and G.W. Wood. 1982a. Identification of diacylglyceryl-*O*-(*N*, *N*, *N*-trimethyl)homoserine in the halotolerant alga, *Dunaliella parva*. Chem. Phys.

Lipids **31**: 331-338.

————, ————, M. GINZBURG AND B.-Z. GINZBURG. 1982b. Lipid composition of halotolerant algae, *Dunaliella parva* Lerche and *Dunaliella tertiolecta*. Biochim. Biophys. Acta **712**: 186-195.

FRIED, A., A. TIETZ, A. BEN-AMOTZ AND W. EICHENBERGER. 1982. Lipid composition of the holotolerant alga, *Dunaliella bardawil*. Biochim. Biophys. Acta **713**: 419-426.

FURLONG, S.T., J.A. LEARY, C.E. COSTELLO AND E.A. DAWIDOWICZ. 1986. Isolation and identification of 1(3), 2-diacylglyceryl-(3)-*O*-4′-(*N*, *N*, *N*-trimethyl)homoserine from the soil amoeba, *Acanthamoeba castellanii*. J. Lipid Res. **27**: 1182-1189.

GIROUD, C., A. GERBER AND W. EICHENBERGER. 1988. Lipids of *Chlamydomonas reinhardtii*. Analysis of molecular species and intracellular site(s) of biosynthesis. Plant Cell Physiol. **29**: 587-595.

GURR, M.I. 1971. The biosynthesis of polyunsaturated fatty acids in plants. Lipids **6**: 266-273.

HARWOOD, J.L. AND A.L. JONES. 1989. Lipid metabolism in algae. Adv. Bot. Res. **16**: 1-53.

JANERO, D.R. AND R. BARRNETT. 1982. Isolation and characterization of an ether-linked homoserine lipid from the thylakoid membrane of *Chlamydomonas reinhardtii* 137$^+$. J. Lipid Res. **23**: 307-316.

KATES, M. 1972. Techniques of lipidology. North-Holland Publishing Co., Amsterdam.

MENDIOLA-MORGENTHALER, L., W. EICHENBERGER AND A. BOSCHETTI. 1985. Isolation of chloroplast envelopes from *Chlamydomonas*. Lipid and polypeptide composition. Plant Sci. **41**: 97-104.

MOSELEY, K.R. AND G.A. THOMPSON, Jr. 1980. Lipid composition and metabolism of *Volvox carteri*. Plant Physiol. **65**: 260-265.

NICHOLS, B.W. 1970. Comparative lipid biochemistry of photosynthetic organisms. *In* J.B. Harborne, ed., Phytochemical Phylogeny, pp. 105-118. Academic Press, London.

———— AND R.S. APPLEBY. 1969. The distribution and biosynthesis of arachidonic acid in algae. Phytochemistry **8**: 1907-1915.

ROUGHAN, P.G. AND C.R. SLACK. 1982. Cellular organization of glycerolipid metabolism. Annu. Rev. Plant Physiol. **33**: 97-132.

SATO, N. 1988. Dual role of methionine in the biosynthesis of diacylglyceryltrimethylhomoserine in *Chlamydomonas reinhardtii*. Plant Physiol. **86**: 931-934.

————. 1991a. Lipids in *Cryptomonas* CR-1. I. Occurrence of betaine lipids. Plant Cell Physiol. **32**: 819-825.

————. 1991b. Lipids in *Cryptomonas* CR-1. II. Biosynthesis of betaine lipids and galactolipids. Plant Cell Physiol. **32**: 845-851.

———— AND M. FURUYA. 1983. Isolation and identification of diacylglyceryl-*O*-4′-(*N*, *N*, *N*-trimethyl)homoserine from the fern *Adiantum capillus-veneris* L. Plant Cell Physiol. **24**: 1113-1120.

———— AND M. FURUYA. 1984a. Distribution of diacylglyceryltrimethylhomoserine in selected species of vascular plants. Phytochemistry **23**: 1625-1627.

———— AND ————. 1984b. The composition of lipids and fatty acids determined at various stages of haploid and diploid generations in the fern *Adiantum capillus-veneris*. Physiol. Plant. **62**: 139-147.

———— AND ————. 1985. Distribution of diacylglyceryltrimethylhomoserine and phosphatidylcholine in non-vascular green plants. Plant Sci. **38**: 81-85.

———— AND K. KATO. 1988. Analysis and biosynthesis of diacylglyceryl-*N*, *N*, *N*-trimethylhomoserine in the cells of *Marchantia* in suspension culture. Plant Sci. **55**: 21-25.

———— AND N. MURATA. 1991. Transition of lipid phase in aqueous dispersions of diacylglyceryltrimethylhomoserine. Biochim. Biophys. Acta **1082**: 108-111.

————, Y. SEYAMA AND N. MURATA. 1986. Lipid-linked desaturation of palmitic acid in monogalactosyl diacylglycerol in the blue-green alga (cyanobacterium) *Anabaena variabilis* studied in vivo. Plant Cell Physiol. **27**: 819-835.

———, Y. NEMOTO AND M. FURUYA. 1988. Lipids of *Chattonella antiqua* (Raphidophyceae). Plant Physiol. Biochem. **26**: 93-98.

SCHLAPFER, P. AND W. EICHENBERGER. 1983. Evidence for the involvement of diacylglyceryl(*N*, *N*, *N*-trimethyl)homoserine in the desaturation of oleic and linoleic acids in *Chlamydomonas reinhardi* (Chlorophyceae). Plant Sci. Lett. **32**: 243-252.

SCHMIDT, H. AND E. HEINZ. 1990. Involvement of ferredoxin in desaturation of lipid-bound oleate in chloroplasts. Plant Physiol. **94**: 214-220.

SHEFFER, M., A. FRIED, H.E. GOTTLIEB, A. TIETZ AND M. AVRON. 1986. Lipid composition of the plasma-membrane of the halotolerant alga, *Dunaliella salina*. Biochim. Biophys. Acta **857**: 165-172.

SIEBERTZ, H.P. AND E. HEINZ. 1977. Labelling experiments on the origin of hexa- and octadecatrienoic acids in galactolipids from leaves. Z. Naturforsch. Series C. **32**: 193-205.

SMITH, K.L. AND J.L. HARWOOD. 1984. Lipids and lipid metabolism in the brown alga *Fucus serratus*. Phytochemistry **23**: 2469-2473.

SOMERVILLE, C. AND J. BROWSE. 1991. Plant lipids: metabolism, mutants, and membranes. Science **252**: 80-87.

STYMNE, S. AND L.-A. APPELQVIST. 1978. The biosynthesis of linoleate from oleoyl-CoA via oleoyl-phosphatidylcholine in microsomes of developing safflower seeds. Eur. J. Biochem. **90**: 223-229.

VASKOVSKY, V.E., S.V. KHOTIMCHENKO AND A.A. BENSON. 1991. Identification of diacylglycero-4′-*O*-(*N*, *N*, *N*-trimethyl)-homoserine in mushrooms. Lipids **26**: 254-256.

VOGEL, G. AND W. EICHENBERGER. 1991. Biosynthesis and metabolism of betaine lipids in *Ochromonas danica* (Chrysophyceae). *In* P.J. Quinn and J.L. Harwood, eds., Plant Lipid Biochemistry, Structure and Utilization, pp. 235-237. Portland Press, London.

VOGEL, G., M. WOZNICA, H. GFELLER, C. MULLER, A.A. STAMPFLI, T.A. JENNY AND W. EICHENBERGER. 1991. 1(3), 2-Diacylglyceryl-3(1)-*O*-2′-(hydroxymethyl) (*N*, *N*, *N*-trimethyl)-β-alanine (DGTA): a novel betaine lipid from *Ochromonas danica* (Chrysophyceae). Chem. Phys. Lipids **52**: 99-109.

WEBER, N., D. BERGENTHAL, C.K. KOKATE AND H.K. MANGOLD. 1989. Biologically active ether lipids: incorporation of long-chain precursors into 1(3), 2-diacylglycero-3(1)-*O*-4′-(*N*, *N*, *N*-trimethyl)homoserines and other lipids of *Chlorella fusca*. J. Lipid Mediators **1**: 37-48.

YAMADA, T. AND Y. NOZAWA. 1979. An unusual lipid in the human pathogenic fungus *Epidermophyton floccosum*. Biochim. Biophys. Acta **574**: 433-439.

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