Phytochemistry, Vol. 21, No. 1, pp. 47–50, 1982.
Printed in Great Britain.

0031-9422/82/010047-04 $03.00/0

COMPARATIVE ANALYSIS OF DIACYLGLYCERYL-TRIMETHYLHOMOSERINE IN *OCHROMONAS DANICA* AND IN *CHLAMYDOMONAS REINHARDTII* 137$^+$

DAVID R. JANERO*† and R. BARRNETT

Section of Cell Biology, Yale University School of Medicine, New Haven, CT 06510, U.S.A.

(*Revised received* 12 *May* 1981)

Key Word Index—*Ochromonas danica*; *Chlamydomonas reinhardtii*; homoserine lipid; fatty acids.

Abstract—The fatty acid compositions of the ether lipid 1(3),2-diacylglyceryl-(3)-*O*-4′-(*N*,*N*,*N*-trimethyl)homoserine (DGTS) from *Ochromonas danica* and *Chlamydomonas reinhardtii* 137$^+$ have been determined. The acyl groups of the DGTS from these two algae grown photosynthetically were compared quantitatively within the context of the general cellular fatty acid profiles. The results demonstrate that the DGTS acyl complement is distinctive for each alga and does not merely mirror the respective cellular fatty acid pattern.

INTRODUCTION

The ether lipid 1(3),2-diacylglyceryl-(3)-*O*-4′-(*N*,*N*,*N*-trimethyl)homoserine (DGTS)‡ was first identified in the golden-brown alga *Ochromonas danica*, where it constitutes over 50% of the polar lipid mass in cells grown heterotrophically under reduced light [1]. In studies on the lipids of an arginine-requiring streptomycin-bleached mutant (strain sr_3) of the single-celled green alga *Chlamydomonas reinhardtii* [2] DGTS was detected in mixotrophically grown cells, but at far lower levels than in *O. danica*. We recently [3] isolated DGTS from phototrophic, wild-type *C. reinhardtii* (strain 137$^+$) and showed that it makes up about 13% of the polar acyl lipid mass, along with glycolipid and phospholipid. Furthermore, we have established DGTS as a *bona fide* membrane lipid by showing that about 40% of the *Chlamydomonas* DGTS is located in the alga's photosynthetic lamellae [3].

At the present time, no cells other than those of these two algae are known to contain DGTS, although the lipid biochemistry of a variety of plant tissues has been detailed [4]. Aside from any importance of DGTS to thylakoid membrane structure and function, inquiry into the relationships among DGTS and the biochemistry, photosynthetic functioning, and evolution of the algae demands quantitative comparison between DGTS of *Ochromonas* and of *Chlamydomonas* grown under favourable photosynthetic conditions. The limited amount of published data on *Ochromonas* DGTS [1] indicates that about 80% of its fatty acids can be accounted for by 14:0, 18:1, and 18:2, whilst our data [3] for the wild-type and the data of others [2] for a mixotrophic mutant strain demonstrate that C_{16} and C_{18} acids dominate in *Chlamydomonas* DGTS. The use of different strains of a given species and variations in, for example, lipid analyses and the extent of quantitation among these studies preclude reliable assessment on their basis of the extent to which DGTS in *Ochromonas* and *Chlamydomonas* are similar. Furthermore, in none of these studies were the algae grown phototrophically.

In this investigation, cellular and DGTS fatty acids of photosynthetic cells of *Ochromonas* have been analysed under conditions identical to those used [3] in a parallel analysis of the fatty acids of cellular lipid and DGTS of phototrophic *Chlamydomonas*. The results are sufficiently detailed to allow molecular comparison between the DGTS of these two phylogenetically distinct algae within the context of their cellular acyl profiles.

RESULTS

Fatty acids of Ochromonas *cellular lipid and DGTS*

The major fatty acids of total lipid and DGTS are quantitated in Table 1. A range of chain lengths, from C_{14} to C_{24}, are present in both cases, with C_{18} and C_{20} acids predominating to comprise some 70% of the acyl complements. C_{14} fatty acids are prominent only in DGTS and are negligible in the cell as a whole. Significant ($P < 0.05$) quantitative differences exist in all chain-length classes common to total lipid and DGTS: algal lipid as a whole has the greater proportion of C_{18} and C_{20} groups, while DGTS is richer in C_{16} and C_{22} fatty acids.

There is a paucity of saturated fatty acids in total lipid relative to that of DGTS, in which about 45% of the acyl groups are saturated. The difference is strikingly illustrated by comparing the unsaturated/saturated ratio at the two levels (Table 1): cellular lipid as a whole is some 14-fold less saturated than DGTS alone. Since there are no significant differences in the amounts of monoenes and

* Present address: Department of Physiological Chemistry, The Johns Hopkins School of Medicine, Baltimore, MD 21205, U.S.A.

† To whom all correspondence should be addressed.

‡ Abbreviation: DGTS, 1(3),2-diacylglyceryl-(3)-*O*-4′-(*N*,*N*,*N*-trimethyl)homoserine.

Table 1. Major fatty acids of whole cells and DGTSs of *O. danica* and *C. reinhardtii* 137$^+$

	Mol % ± s.d. (*O. danica*, 4; *C. reinhardtii*, 12)			
	O. danica		*C. reinhardtii*	
Fatty acid	Total cellular lipid	DGTS	Total cellular lipid	DGTS
14:0	< 1.0	20.69 ± 1.18	< 1.0	< 1.0
16:0	2.84 ± 0.41	3.65 ± 0.12	14.36 ± 0.39	26.49 ± 0.89
16:1	2.77 ± 0.23	< 1.0	< 1.0	2.90 ± 0.14
16:2	nd*	nd*	1.14 ± 0.05	2.15 ± 0.27
16:3	< 1.0	4.59 ± 0.24	3.59 ± 0.11	1.38 ± 0.16
18:0	< 1.0	< 1.0	2.98 ± 0.09	3.72 ± 0.18
18:1	7.37 ± 0.21	11.49 ± 0.50	10.40 ± 0.22	6.90 ± 0.48
18:2	4.63 ± 0.91	16.40 ± 0.58	11.99 ± 0.26	7.55 ± 0.22
18:3	12.19 ± 1.75	1.89 ± 0.15	31.09 ± 0.93	2.34 ± 0.16
18:4	15.91 ± 1.47	< 1.0	6.79 ± 0.21	16.25 ± 1.59
20:0	2.57 ± 0.12	9.90 ± 0.17	4.56 ± 0.40	1.44 ± 0.09
20:1	3.88 ± 0.25	1.14 ± 0.13	2.91 ± 0.20	12.27 ± 0.87
20:2	5.53 ± 0.31	2.09 ± 0.24	1.09 ± 0.15	6.52 ± 0.57
20:3	12.16 ± 0.35	2.10 ± 0.01	< 1.0	< 1.0
20:4	8.13 ± 0.64	8.19 ± 0.20	< 1.0	< 1.0
22:0	< 1.0	10.35 ± 0.89	nd*	nd*
22:1	2.12 ± 0.17	3.05 ± 0.20	< 1.0	< 1.0
22:2	3.52 ± 0.16	< 1.0	2.45 ± 0.31	2.07 ± 0.07
22:3	2.87 ± 0.10	< 1.0	nd*	nd*
24:2	3.63 ± 0.35	< 1.0	nd*	nd*
Sum:	90.08 %	95.53 %	93.46 %	91.98 %
Unsaturated/saturated:	16.27 ± 1.21	1.19 ± 0.09	3.21 ± 0.22	1.98 ± 0.14

Fatty acids comprising > 1.0 mol % of the total algal and DGTS fatty acid complements are quantitated. The sum of the tabulated mol % is indicated for each group, as is the ratio of unsaturated/saturated fatty acids.

* nd, None detected.

dienes, the highly unsaturated character of the alga's total lipid is due to the relative lack of saturated acyl groups coupled to enrichment in trienes and tetraenes.

These differences in acyl chain-length distribution and saturation between total *Ochromonas* cellular lipid and the alga's DGTS are reflected in the contrasting profiles of individual major fatty acids (Table 1). The predominant cellular fatty acid, 18:4, is not a major DGTS fatty acid, and vice versa with respect to DGTS 14:0. Of the major fatty acids which are common to both lipid classes, only one, 20:4, is found at the same amount in total algal lipid and in DGTS. The basis for the greater unsaturation of total *Ochromonas* lipid relative to DGTS lies in all but two (16:0, 20:0) of its major fatty acids being unsaturated, while DGTS has four major saturated fatty acids (including its predominant fatty acid, 14:0). The homoserine lipid has a relatively small range of fatty acids, since about 90% of the DGTS acyl complement, but only about 75% of the total cellular fatty acids can be accounted for by the 10 most abundant components.

Fatty acids of Chlamydomonas *cellular lipid and DGTS*

For comparative purposes, the major fatty acids of cellular lipid and DGTS are also presented in Table 1, along with the unsaturated/saturated ratio at both levels. In both cases, some 13 fatty acids comprise over 90% of the total found. The cellular lipid and DGTS contain mainly C_{16} and C_{18} acyl groups [3] and have 12 of the 13 major fatty acids in common (16:1 the exception). Quantitatively, however, the fatty acid complement of DGTS is distinct from that of the cell as a whole, with 16:0 predominant in the lipid and 18:3 in the alga. The total algal lipid has a significantly higher unsaturated/saturated ratio, due principally to the prevalence of C_{18} unsaturated fatty acids.

DISCUSSION

Parallel analyses of the fatty acids of DGTS from photosynthetically cultured *Ochromonas* and *Chlamydomonas* were carried out to establish a quantitative basis by which comparison between these two algal cells could be made. To provide an appropriate context for comparison at the individual lipid level, the cellular complements of fatty acids from the two algae were also analysed.

The results obtained on the fatty acids of *Ochromonas* serve to extend the limited published information and support the finding of Nichols and Appleby [5] that *Ochromonas* contains a wide range of acyl chain lengths. However, these authors did not detect any C_{24} acids and found greater amounts of short-chain saturated fatty acids (e.g. 14:0, 16:0) and fewer longer-chain polyunsaturated fatty acids (e.g. 18:4, 20:3) in their

mixotrophic cells. Our data regarding *Ochromonas* DGTS are in agreement with the prevalence of 14:0, 18:1, and 18:2 noted in the only other study of this lipid [1], while we have detected and quantitated some major long-chain fatty acids (e.g. 20:0, 20:4, 22:0, 22:1) not observed in that earlier investigation, which utilized *Ochromonas* grown heterotrophically.

Such comparison among various studies is tenuous at best, for a number of reasons, including the fragmentary nature of the data itself, the use of differing strains within a genus, the use of cultures which are in a stationary growth phase, etc. Growth under conditions detrimental to photosynthesis is also complicating, since for at least some algae (*Euglena* [6] and *Chlorella* [7]) evidence exists to suggest that poor growth conditions alter the cellular fatty acid complement. Further, the culturing of *Ochromonas* [8] or a yellow mutant of *Chlamydomonas* (strain y-1 [9]) in the dark causes changes in the cellular acyl profiles (with respect to cells grown photosynthetically) concomitant with a loss of thylakoid membrane.

We have sought to obviate complications from light deprivation, etiolation, and the dependence of cellular anabolism on external carbon sources by making comparisons between log-phase *Ochromonas* and *Chlamydomonas* cultured under conditions favourable to their active photosynthetic growth. Qualitatively, eight major fatty acids are common to DGTS from the two algae (16:0, 16:3, 18:1, 18:2, 18:3, 20:0, 20:1, and 20:2). None of these eight fatty acids is present in the same amount in both cells, however. *Chlamydomonas* DGTS is almost twice as unsaturated as *Ochromonas* DGTS, while the former contains more shorter-chain C_{16} and C_{18} acids and less of the longer-chain C_{20} and C_{22} varieties.

The differences between *Ochromonas* and *Chlamydomonas* DGTS become even more striking when placed in the context of the fatty acid profiles of the cells as a whole, as expected from the generally distinctive acyl complements which characterize individual plant tissue lipids [4, 10]. Despite certain qualitative similarities in the mutual presence of individual fatty acids (e.g. 16:0, 18:1), *Ochromonas* DGTS has such a distinctive acyl complement with respect to the alga's total fatty acid profile that its unsaturated/saturated ratio is some 14-fold less. *Chlamydomonas* DGTS fatty acids are also quantitatively distinct from and more saturated than those of the total cell lipid. Since DGTS from either alga does not reflect to any great quantitative extent the general cellular fatty acid profile, we conclude that DGTS from these two phylogenetically distinct algae grown phototrophically are very different in molecular terms which do not simply mirror the dissimilar acyl profiles of the respective cells. To provide a physiological basis for these differences, we are currently investigating the fatty acid synthesizing and acyl-transferase properties of the two algae and any variations therein which may be induced by altered photosynthetic metabolism.

EXPERIMENTAL

Cell culture. Ochromonas danica Pringsheim (University of Texas Algal Culture Collection, UTEX-1298) was grown phototrophically in the medium described by [11] (pH 6.8), modified by the substitution of NH_4NO_3 (0.3 g/l) for NH_4Cl, and of EDTA as chelator for nitrilotriacetic acid [12], and by the use of the trace metals solution of ref. [13] supplemented with $FeCl_3 . 6H_2O$ (0.2 mg) and $Na_3VO_4 . 16H_2O$ (1.0 μg). *Chlamydomonas reinhardtii* 137^+ was cultured phototrophically and asynchronously in minimal medium [13]. Both algae were grown axenically at 25 ± 0.5° and in 7.7 klx of white fluorescent light with continuous shaking and bubbling with a moistened gas mixture [9]. Cells were harvested during logarithmic phase by low-speed centrifugation; growth was monitored by haemacytometer counting of fixed [14] cells.

Purification of cellular DGTS. Algal lipids were quantitatively extracted by a modification of the method of ref. [15]. Purification of DGTS from *Ochromonas* was carried out chromatographically [1]. *Chlamydomonas* lipids were sepd by TLC with the solvents of ref. [16] on 0.25 mm Si gel F. Resolved lipids were visualized under UV radiation; DGTS $R_f(x, y)$ 0.61, 0.52. The final DGTS spots whose fatty acids were analysed each gave a single H_2O-soluble deacylation product after mild alkaline hydrolysis [17]. Both the intact *Ochromonas* and *Chlamydomonas* DGTSs and their H_2O-soluble hydrolysis products co-migrated in a number of solvents on paper and Si gel [18] and showed typical, positive ninhydrin and Dragendorff staining [1].

Fatty acid analysis. Portions of total algal lipid extracts or adsorbent containing DGTS recovered from the TLC support were quantitatively transesterified in 0.5 N MeONa [19]. Fatty acid methyl esters were separated by GLC on a 10% stabilized diethylene glycol succinate glass column (Supelco, Bellefonte, PA) in an HP 5830A gas chromatograph (Hewlett–Packard, Chicago, IL) operated isothermally at 200° with carrier N_2 (25 ± 1 ml/min). In the cases where total cell lipid was exposed to alcoholic base, fatty acid methyl esters were purified from saponifiable pigment derivatives [20] by TLC [21] prior to GLC. Total fatty acids were, in some experiments, further fractionated into subclasses based on unsaturation by Ag^{2+} TLC [22] prior to GLC. All GLC analyses were carried out well within the linear response range of the detector as calibrated with standard fatty acid methyl ester mixtures.

Fatty acids were identified on the basis of combined information from several sources, principally R_fs of known commercial ester standards and mathematical analyses of R_f–chain length relationships under the conditions employed [cf. 23]. Quantitation of peak areas on GLC chromatograms was through digital integration, and conversion of relative areas to mol-per cent composition was based on response factors obtained with the quantitative standards [23].

Statistical analysis. Statistical evaluation of the difference between two means was made with a Student's *t*-test [24]; the significance level was set at the ninety-fifth confidence interval, $P < 0.05$ indicating a statistically significant difference.

Acknowledgements—This work was supported in part by Research Grant AM-03688 from the U.S. Public Health Service. D.R.J. was a Predoctoral Fellow supported by National Institutes of Health Grant GM-07223. We thank Drs. P. Fletcher, G. Davis, and R. Paradise, Centre for the Study of Cellular Membranes, Yale University School of Medicine, for the use of their GLC equipment, and Ms. L. LaGreca for her secretarial assistance.

REFERENCES

1. Brown, A. E. and Elovson, J. (1974) *Biochemistry* **13**, 3476.
2. Eichenberger, W. and Boschetti, A. (1978) *FEBS Letters* **88**, 201.
3. Janero, D. R. and Barrnett, R. (1979) *J. Cell Biol.* **83**, 360a.
4. Harwood, J. L. (1979) *Prog. Lipid Res.* **18**, 55.
5. Nichols, B. W. and Appleby, R. S. (1969) *Phytochemistry* **8**, 1907.

6. Pohl, P. and Wagner, H. (1972) *Z. Naturforsch. Teil B* **27**, 53.
7. Pohl, P., Passig, T. and Wagner, H. (1971) *Phytochemistry* **10**, 1505.
8. Haines, T. H., Aaronson, S., Gellerman, J. L. and Schlenk, H. (1962) *Nature* **194**, 1282.
9. Goldberg, I. and Ohad, I. (1970) *J. Cell Biol.* **44**, 563.
10. Kirk, J. T. O. and Tilney-Bassett, R. A. E. (1978) in *The Plastids*, pp. 50–63. Elsevier, Amsterdam.
11. Aaronson, S. and Baker, H. (1959) *J. Protozool.* **6**, 282.
12. Hutner, S. H., Baker, H., Aaronson, S., Natan, H. A., Rodriquez, E., Lockwood, S., Sanders, M. and Peterson, R. (1954) *J. Protozool.* **4**, 259.
13. Sager, R. and Granick, S. (1953) *Ann. N. Y. Acad. Sci.* **56**, 831.
14. Bourguignon, L. Y. W. and Palade, G. E. (1976) *J. Cell. Biol.* **69**, 327.
15. Marshall, M. O. and Kates, M. (1972) *Biochim. Biophys. Acta* **260**, 558.
16. Allen, C. F. and Good, P. (1971) *Methods Enzymol.* **23**, 523.
17. Brockerhoff, H. (1963) *J. Lipid Res.* **4**, 96.
18. Benson, A. A. and Mauro, B. (1958) *Biochim. Biophys. Acta* **27**, 189.
19. Marinetti, G. V. (1966) *J. Lipid Res.* **7**, 786.
20. Suyama, K., Hori, K. and Adachu, S. (1979) *J. Chromatogr.* **174**, 234.
21. Morrison, W. R. and Smith, L. M. (1964) *J. Lipid Res.* **5**, 600.
22. Morris, L. J. (1966) *J. Lipid Res.* **7**, 717.
23. Ozcimder, M. and Hammers, W. E. (1980) *J. Chromatogr.* **187**, 307.
24. Bishop, O. N. (1966) in *Statistics for Biology*, p. 130. Houghton Mifflin, Boston.