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OCTADECAPENTAENOIC ACID IN A RAPHIDOPHYTE ALGA, *HETEROSIGMA AKASHIWO*

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Abstract—Total fatty acids from *Heterosigma akashiwo* contained 4.8–8.9 wt% of octadecapentaenoic acid (18:5n-3) through the growth cycle. This polyunsaturated fatty acid was preferentially located in monogalactosyldiacylglycerols and digalactosyldiacylglycerols (20.1% and 10.2% of fatty acids, respectively, at day 18) with 0.3% or less in sulphoquinovosylglycerol (SQDG)+phosphatidylglycerol (PG) and phosphatidylcholine. The SQDG+PG fraction contained six *cis* 16:1 isomers. These findings are discussed in relation to algal taxonomy. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Octadecapentaenoic acid (18:5n-3) is an unusual fatty acid confined to certain classes of marine algae. It was first identified in dinomastigote algae by Joseph [1], which led Mazaud [2] to suggest that its restricted distribution could be a useful marker in the marine food web. It was subsequently found also in Prymnesiophytes [3], which led Okuyama *et al.* [4] to hypothesise that Prymnesiophyte endosymbionts could account for 18:5n-3 in dinoflagellates. However, Nichols *et al.* [5] had previously found 18:5n-3 in Australian and British strains of the raphidophyte alga, *Heterosigma akashiwo*, although this study was not cited by subsequent authors, e.g. Dunstan *et al.* [6], when reporting 18:5n-3 in several Prasinophyte algae; its significance would appear to have been overlooked. In earlier studies, 18:5n-3 was probably mis-identified as 20:1n-9, since on polar GC columns these fatty acids coelute. The distribution of 18:5n-3 may, therefore, be more widespread than believed. Here, we report the fatty acid composition of the total lipids of *H. akashiwo* during the growth cycle and the fatty acid profiles of its main polar lipid classes.

RESULTS

The fatty acid composition of total lipids and of the main polar lipid classes, phosphatidylcholine (PC), digalactosyldiacylglycerol (DGDG), monogalactosyldiacylglycerol (MGDG) and sulphoquinovosylglycerol (SQDG)+phosphatidylglycerol (PG), was determined after 6 and 10 days (log-phase), 14 and 18 days (late log-phase) and 22 days (stationary phase). In total lipids, polyunsaturated fatty acids (PUFA), predominantly n-3 PUFA, comprised up to 59.1% of the fatty acids, peaking in late log-phase (day 14) (Table 1). Eicosapentaenoic acid (20:5n-3) and 18:4n-3 were the main components, but 18:5n-3 comprised 4.8–8.9% of the total fatty acids, decreasing during growth, with 18:3n-3 and 22:6n-3 comprising 3.3–4.7% and 3.8–5.2%, respectively (Table 1). Saturated fatty acids (SFA), predominantly 16:0, totalled 34.8% of fatty acids, decreasing during the log-phase and peaking in the stationary phase. Monounsaturated fatty acids (MUFA) showed a slight increase during growth, 16:1n-7 being the largest component (6.2–10.7%) (Table 1).

The fatty acid composition of the four lipid classes was very similar for days 6, 10, 14 and 18, while more SFA and less PUFA was present in each class on day 22 (data not shown), reflecting changes in the total lipids (Table 2). In PC, 16:0 and 20:5n-3 were the main fatty acids, with 22:6n-3 contributing up to 11.5% at day 18 (Table 2). The C_{18} PUFA, 18:2n-6, 18:3n-3 and 18:4n-3, each contributed up to 5%, while 18:5n-3 was a minor component of PC (<1%) (Table 2). Almost all of the 20:4n-6 and 20:4n-3, up to 1.4% and 3.0%, respectively, was found in the PC (data not shown). PUFA dominated DGDG, increasing during growth from 71.5% to 86.0% (data not shown) with 79.2% at day 18 (Table 2). These were predominantly 18:4n-3 and 20:5n-3 (32.1% and 31.2%, respectively, at day 18) but 18:5n-3 contributed 10.2% and 18:3n-3 4.0% (Table 2). Although PUFA were also major constituents of MGDG (total

Table 1. Fatty acid composition (wt%) of total lipids from *H. akashiwo* with growth

Fatty acid	Day 6	Day 10	Day 14	Day 18	Day 22
14:0	5.9	4.9	4.8	6.4	6.5
15:0	0.6	0.5	0.6	2.5	1.6
16:0	23.1	18.1	17.5	23.3	26.4
18:0	0.8	0.4	0.5	1.3	0.3
Total SFA	30.4	23.9	23.4	33.5	34.8
16:1n-9	0.7	0.4	0.5	0.7	0.3
16:1n-7	6.2	7.5	7.8	8.6	10.7
16:1n-11+n-5	1.2	2.0	2.2	2.3	2.6
16:1n-4	0.5	0.9	0.8	0.8	0.7
16:1n-13 *trans*	1.9	2.3	2.1	1.3	0.7
16:1n-3	2.6	2.9	2.8	2.5	2.2
18:1n-9	2.0	0.8	1.0	1.7	1.6
18:1n-7	0.3	0.5	0.5	0.6	0.3
Total MUFA	15.3	17.3	17.6	18.5	19.1
16:2n-6	0.4	1.0	1.1	0.9	0.7
18:2n-6	1.1	1.0	0.9	1.1	1.4
20:4n-6	0.3	0.2	0.3	0.2	0.5
Total n-6 PUFA	1.8	2.2	2.3	2.2	2.6
18:3n-3	4.7	3.4	3.3	3.4	4.0
18:4n-3	16.3	16.1	16.5	12.7	11.0
18:5n-3	8.9	7.7	8.0	6.0	4.8
20:4n-3	0.6	0.6	0.4	0.4	0.8
20:5n-3	17.6	23.3	23.5	18.6	18.8
22:5n-3	0.3	0.3	0.3	0.9	0.3
22:6n-3	4.0	5.2	4.8	3.9	3.8
Total n-3 PUFA	52.5	56.6	56.8	45.9	43.5

Data from one culture.

Table 2. Distribution of major fatty acids (wt%) in polar lipid fractions of *H. akashimo* during late log-phase growth (day 18)

Fatty acid	PC	SQDG+PG	DGDG	MGDG
16:0	28.6	26.8	7.6	14.4
Total SFA	34.5	35.6	14.8	23.0
16:1n-7	0.7	5.3	3.0	12.4
16:1n-11+n-5	0.0	6.8	0.0	0.0
16:1n-4	0.0	2.6	0.0	0.0
16:1n-13 *trans*	0.0	4.0	0.0	0.0
16:1n-3	0.0	8.9	0.0	0.0
18:1n-9	1.6	1.3	4.2	1.8
Total MUFA	4.9	30.4	6.0	14.9
18:2n-6	2.9	0.7	0.8	0.8
18:3n-3	2.9	4.0	4.0	2.9
18:4n-3	1.6	4.6	32.1	27.6
18:5n-3	0.0	0.2	10.2	20.1
20:5n-3	37.6	20.9	31.2	9.5
22:6n-3	11.5	2.8	0.0	0.3
Total PUFA	60.5	34.1	79.2	62.1

Data from one culture.

ca 60%), the fatty acid profile was different with much less 20:5n-3 (9.5% at day 18), more 18:5n-3 (20.1%) and a similar amount of 18:4n-3 (27.6%). Small amounts of 18:2n-6 and 18:3n-3 were present and 22:6n-3 was absent from both galactolipids. Palmitic acid (*ca* 15%) was the other main fatty acid in MGDG and 16:1n-7 was the major MUFA.

In the SQDG+PG fraction, 20:5n-3 (20.9% at day 18) was the predominant PUFA with lesser amounts of 18:3n-3 (4.0%), 18:4n-3 (4.6%) and 22:6n-3 (2.8%); there was less than 1% 18:2n-6 and 18:5n-3 (Table 2). SFAs were equally or more abundant than PUFA with 16:0 comprising 26.8% and MUFA 30.4% of the fatty acids at day 18 (Table 2). Of particular interest, were seven 16:1 isomers, five of which (n-11, n-5, n-4, n-3 and n-13-*trans*) were only present in the SQDG+PG fraction. The 16:1 acids accounted for up to 31.3% of the fatty acids at day 14 (data not shown). However, we were unable to resolve 16:1n-11 and 16:1n-5 on either BP20 or CP SIL 5 columns.

A replicate culture grown from a separate sub-stock showed a very similar fatty acid composition with 5.9% 18:5n-3 in the total lipids at day 18 (21.9% and 12.8%, respectively, in MGDG and DGDG)

and 29.0% 16:1 isomers in SQDG+PG (data not shown).

DISCUSSION

The presence of 18:5n-3 in a raphidophyte alga [5, this study], together with the discovery of up to 16.7% of the total fatty acid as 18:5n-3 in five out of six Prasinophytes examined [6], established that this acid is more widespread in marine algae than was previously thought, although the bulk of this fatty acid in the marine ecosystem is still likely to be contributed by dominant bloom species, such as Prymnesiophytes and photosynthetic dinoflagellates. 18:5n-3 is probably formed by chain-shortening of 20:5n-3 by analogy with the biosynthesis of 22:6n-3, which occurs in vertebrates by a Δ-6 desaturase acting on 24:5n-3 and subsequent chain-shortening of 24:6n-3 in peroxisomes [7]. This biosynthetic pathway has yet to be demonstrated in algae. However, selectivity of putative chain-shortening in algae must occur, since many species able to synthesise 22:6n-3, and which contain 20:5n-3, have no 18:5n-3. Currently, the relationship between the relative contributions of 18:4n-3, 18:5n-3, 20:5n-3 and 22:6n-3 in different algae is not understood. C_{18} PUFA are largely located in the glycolipids of thylakoid membranes [e.g. 8] but it is unclear what functional benefits accrue from having 18:5n-3 rather than 18:4n-3 or 18:3n-3 in chloroplast glycolipids. In *Emiliania huxleyi*, 18:5n-3 was preferentially found in DGDG [8], whereas in *H. akashiwo* it is present predominantly in MGDG.

The presence of six *cis* 16:1 isomers in the SQDG+PG fraction was also noteworthy and raises interesting questions concerning their biosynthesis and functional significance. A recent study found 16:1n-3 in SQDG from *H. carterae* [9]. C_{16} and C_{18} monoenoic fatty acids, other than the n-7 and n-9 isomers, are usually only found in prokaryotes. It is notable that the small subunit of ribulose-1,5-bisphosphate carboxylase (RUBISCO) from *Olisthodiscus luteus* (probably mis-identified and believed to be *H. akashiwo* [10]) showed greatest genomic similarity to the chemolithotrophic bacterium, *Alcaligenes eutrophus* [11]. However, it is possible to account, theoretically, for the synthesis of 16:1n-3, n-4, n-5 and n-11 from 12:0, 14:0 and 16:0 using Δ-5, Δ-9 or Δ-12 desaturases, all of which are generally present in photosynthetic algae.

Dinoflagellates are thought to have acquired chloroplasts from various other algal groups [12]. However, the fact that all photosynthetic dinoflagellates so far examined contain 18:5n-3, suggests that their chloroplasts came from a restricted group of algae containing 18:5n-3, *viz*., Prymnesiophytes, Prasinophytes or Raphidophytes. In the Prymnesiophytes, 18:5n-3 is absent from the Pavlovales [13], but abundant in other orders. The variable amounts of 18:5n-3 in dinoflagellates (3–34% of total fatty acid [1, 4]) may be indicative of different initial endosymbionts. Detailed profiling of the fatty acids and photosynthetic pigments of chloroplasts from dinoflagellates and possible donor algae will help in elucidating these relationships.

EXPERIMENTAL

Growth of alga.

H. akashiwo (Hada) (PML culture no. 239) was grown in 1 l of F/2 media at 15° under a 12 hr light–dark cycle (100 μE sec^{-1}) using 75 W fluorescent tubes. Growth was monitored at 2 day intervals using a flow-through Coulter counter. Aliquots (100 ml) of culture were harvested for lipid analysis by filtering through Whatman GF/F filters ashed at 500°. Filtered algae were placed in 7 ml $CHCl_3$–*iso*PrOH (2:1) containing 0.01% butylated hydroxytoluene and stored at −20° under N_2 for at least 19 days before lipid extraction.

Lipid extraction and analysis. Solvent was removed and the filter rinsed with a further 7 ml $CHCl_3$–*iso*-PrOH (2:1). The extract was homogenised, filtered and evapd to dryness under N_2. A Folch extract of total lipid was then prepd [14]. Fatty acid Me esters (FAME) of total lipids and main polar lipid classes (PC, SQDG+PG, DGDG and MGDG) were prepd as described previously [8] using 1% H_2SO_4 in MeOH for 16 hr under N_2 at 50°. After extraction and purification, FAME were analysed using a BP20 fused silica capillary column (50 m × 0.32 mm i.d.) (SGE) using H_2 as carrier [8]. Peaks were identified by reference to samples of known composition and by GC-MS using a DB-5MS column (15 m × 0.25 mm i.d.) (J & W Scientific) with He as carrier gas. An authentic sample of 18:5n-3 obtained from *Emiliania huxleyi* [8] was available for comparison. The GC-MS was operated in EI+*ve* and CI+*ve* modes for FAME, and EI+*ve* mode for fatty acid diethylamides [15] and dimethyldisulphide (DMDS) adducts [16]. The diethylamide derivative of 18:5n-3 was identified by *m/z* $[M]^+$ 329 and a 26 *m/z* 300 and *m/z* 274 diagnostic of an n-3 fatty acid [15]. 16:1 isomers suggested by EI and CI fragmentation of FAME were confirmed following $AgNO_3$ TLC of FAME from the SQDG+PG band to give *cis*- and *trans*-monoene frs and prepn of DMDS adducts [16]. 16:1 isomers of DMDS adducts were characterised by *m/z* $[M]^+$ 362 and diagnostic ω- and δ-fragments giving the double bond position [16].

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