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POSITIONAL DISTRIBUTION OF FATTY ACIDS IN LIPIDS OF THE MARINE DIATOM *PHAEODACTYLUM TRICORNUTUM*

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Key Word Index—*Phaeodactylum tricornutum*; marine diatom; positional distribution of fatty acids; eicosapentaenoic acid; lipid; monogalactosyldiacylglycerol; phosphatidylcholine.

Abstract—The composition of fatty acids and lipids in the marine diatom, *Phaeodactylum tricornutum* was determined. The lipids consisted of monogalactosyldiacylglycerol, digalactosyldiacylglycerol, sulphoquinovosyldiacylglycerol, phosphatidylglycerol, phosphatidylcholine, phosphtidylinositol, triacylglycerol and minor unidentified ones. At the early stationary phase of growth, the total fatty acids were mainly 20:5, 16:1, 16:0 and 16:3. 20:5 was distributed in polar lipids, particularly in monogalactosyldiacylglycerol, phosphatidylcholine and phosphatidylglycerol. This fatty acid was exclusively located at the *sn*-1 position of the glycerol moiety in all polar lipids except for phosphatidylcholine. In phosphatidylcholine 20:5 was distributed at both the *sn*-1 and *sn*-2 positions. 16:3 was concentrated at the *sn*-2 position of monogalactosyldiacylglycerol and *trans*-16:1 (*n*-13) was dominant at the *sn*-2 position of phosphatidylglycerol. C_{18} fatty acids, the minor fatty acids in *P. tricornutum*, were confined to the *sn*-2 position of phosphatidylcholine.

INTRODUCTION

C_{20} polyunsaturated fatty acids are the characteristic fatty acids in many of Rodophyceae, Phaeophyceae, Chrysophyceae, Haptophyceae, Bacillariophyceae, Xantophyceae and Chlorophyceae [1]. In *Porphyra yezoensis* and *Porphyra tenera*, 20:5 comprises 53% and 40% of the total fatty acids, respectively [2, 3]. In *Fucus serratus* 20:5 was 8% and 20:4 was 10% of total fatty acids [4]. In diatoms the content of 20:5 was also high; 18% in *Phaeodactylum tricornutum* [5] and 17% in *Navicula incerta* [6].

A large quantity of 20:5 was found in glycolipids from the red alga *Porphyra tenera* [3] and the brown alga *Laminaria angustata* [7], and in all lipid classes from the red alga *Porphyra yezoensis* [2] and the diatom *N. incerta* [6].

The positional distribution of 20:5 in lipid classes has not been elucidated; it is interesting in relation to the biosynthetic pathway of this acid. This paper reports analysis of lipids from *Phaeodactylum tricornutum* with special respect to the positional distribution of 20:5 in its constituent lipid classes.

RESULTS

The fatty acid composition of *P. tricornutum* cells at lag, logarithmic and early stationary phases of growth is shown in Table 1. At lag phase the major fatty acids were 16:0, 16:1 and 18:1; 20:5 accounted for only 6% of the total fatty acids. At logarithmic phase the major fatty acids were 16:0, 16:1 and 20:5 as the result of a marked increase of 20:5 and a marked decrease of 18:1. At stationary phase, the main fatty acids were 16:0, 16:1, 16:3 and 20:5, the percentage of 20:5 reaching the highest level among the three growth phases. Thus, the degree of unsaturation of the lipids markedly increased from lag phase to stationary phase through the logarithmic phase.

Table 1. Fatty acid composition of total lipids from *P. tricornutum*

Fatty acid*	Fatty acid composition (molar %)		
	Lag phase	Logarithmic phase	Stationary phase
14:0	5.8	8.8	6.8
16:0	26.7	20.0	14.5
16:1 (*n*-7) + 16:1 (*n*-13)†	34.2	24.5	27.2
16:2	1.3	3.2	3.8
16:3	2.9	6.1	13.4
18:0	2.7	2.7	0.7
18:1	20.0	8.8	2.8
18:2	0	3.6	3.0
20:5	6.4	22.3	27.8

*Fatty acids are designated by the number of carbon atoms: number of double bonds. Figures in parentheses show the position of the first double bond from the methyl end of the carbon chain.

The lipids of *P. tricornutum* consisted of monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), sulphoquinovosyldiacylglycerol (SQDG), phosphatidylglycerol (PG), phosphatidylcholine (PC), phosphatidylinositol (PI), triacylglycerol (TG) and minor unidentified ones. The ratio of the main polar lipids to MGDG in the cells at early stationary phase was MGDG 1:DGDG 0.30:SQDG 0.34:PG 0.17:PC 0.24.

The fatty acid composition of the individual lipids of *P. tricornutum* at early stationary phase is shown in Table 2.

Table 2. Fatty acid composition of the major lipids from early stationary phase cells of *P. tricornutum*

Fatty acid	Fatty acid composition (molar %)					
	MGDG	DGDG	SQDG	PG	PC	TG
14:0	1.9	6.6	33.9	1.0	3.8	23.0
16:0	4.1	25.8	23.3	20.5	9.8	18.6
16:1 (*n*-7)	22.2	27.6	30.7	15.6	13.8	23.7
16:1 (*n*-13)*t*	0	0	0	26.4	0	0
16:2	1.9	4.6	2.9	0	1.5	0
16:3	25.4	1.7	1.2	0	0.8	8.2
16:4	3.6	0	0	0	0	0
18:0	0	2.8	0	0	0	12.5
18:1	1.8	4.3	3.0	6.1	9.3	4.8
18:2	0.8	2.3	0.6	1.8	17.0	0
18:3	0.7	1.6	0.2	0.5	2.2	2.7
20:2	0.2	3.1	0	0	0	0
20:3	0.7	0.1	0	0.9	5.6	0
20:4 (*n*-6)	0.6	0.3	0	0	2.8	2.5
20:4 (*n*-3)	2.1	0.5	0	0	2.5	0
20:5	34.0	18.7	5.2	27.2	30.9	4.0

In MGDG, PC and PG, 20:5 comprised *ca* 30% of the total fatty acids, whereas this acid accounted for only 5% of those in SQDG and TG. The other C_{20} fatty acids such as 20:3 and 20:4(*n*-6) were minor components. The content of C_{18} fatty acids was also low in all lipid classes except for 18:0 in TG, and 18:1 and 18:2 in PC. 16:1 (*n*-7) was widely distributed in all lipids, whereas *trans*-16:1(*n*-13) was restricted to PG. 16:3 was predominantly located in MGDG. The main species of saturated fatty acids were 16:0 and 14:0. They were distributed in all lipid classes, 16:0 occurring mainly in PG, DGDG, SQDG and TG, 14:0 in SQDG and TG.

Positional analyses of individual lipids are shown in Tables 3 and 4. 20:5 was located almost exclusively at the *sn*-1 position of all polar lipid classes except for PC. In PC, *ca* 83% of this acid were present at the *sn*-1 position and *ca* 17% at the *sn*-2 position. 16:3 was restricted to the *sn*-2 position of MGDG and *trans*-16:1(*n*-13) was dominant at the *sn*-2 position of PG. 18:1 and 18:2 in PC were also predominantly located at the *sn*-2 position. More than half of 16:1(*n*-7) was found at the *sn*-2 position of all glycolipids. Conversely, 16:1(*n*-7) in PC and PG was present at the *sn*-1 rather than at the *sn*-2 position.

Table 3. Distribution of fatty acids at the *sn*-1 and *sn*-2 positions in the glycolipids from *P. tricornutum*

Fatty acid	Fatty acid composition (molar %)					
	MGDG		DGDG		SQDG	
	sn-1	*sn*-2	*sn*-1	*sn*-2	*sn*-1	*sn*-2
14:0	3.2	0.6	11.9	0.3	62.2	5.6
16:0	0.1	8.1	31.4	17.3	1.5	45.1
16:1 (*n*-7)	17.4	26.8	11.0	48.8	18.5	42.9
16:2	1.5	2.3	1.6	7.5	4.0	0
16:3	3.6	47.2	2.1	1.1	1.4	1.0
16:4	1.2	6.0	0	0	0	0
18:0	0	0	3.9	1.4	0	0
18:1	0.9	2.7	1.4	7.2	1.0	5.0
18:2	0.4	1.2	0	4.7	0.8	0.4
18:3	0.5	0.9	0	6.3	0.4	0
20:2	0	1.7	4.2	1.6	0	0
20:3	0.5	0.9	0	0.2	0	0
20:4 (*n*-6)	1.2	0	0.6	0	0	0
20:4 (*n*-3)	3.8	0.4	0.5	0.5	0	0
20:5	65.7	1.3	31.5	3.1	10.4	0

DISCUSSION

The major fatty acids of the diatom *P. tricornutum* were 20:5, 16:1, 16:3 and 16:0 at early stationary phase of growth (Table 1), in agreement with previous work [5]. Hexadecenoic acid in all lipids except for PG was identified as 16:1 (*n*-7) by GC/MS. In PG a quarter was 16:1 (*n*-7) and three quarters were *trans*-16:1 (*n*-13).

The marine diatom *N. incerta* contains a large amount of 20:5 in all lipid classes, but *P. tricornutum* contained large amounts only in MGDG, PC, PG and DGDG [6]. On the other hand, 20:3, 20:4 (*n*-6) and 20:4 (*n*-3) were found in PC from *P. tricornutum*, but little in any lipid classes from *N. incerta*. Restricted distribution of 16:3 in MGDG from *P. tricornutum* is the same as found in *N. incerta*. There were little C_{18}-fatty acids in lipids from both diatoms except for PC from *P. tricornutum*.

Polyunsaturated fatty acids are mainly C_{18} or both C_{18} and C_{16} in higher plants, but both C_{20} and C_{16} occur in the diatoms *P. tricornutum* and *N. incerta* [6]. Fatty acids were more unsaturated in MGDG and less unsaturated in SQDG in *P. tricornutum*, consistent with those found in higher plants [8]. The lipids of the diatom *P. tricornutum* can be classified into four groups with respect to the positional distribution of fatty acids in the lipids (Table 5).

Roughan *et al.* [9] classified lipids of higher plants, green algae and blue-green algae into two types; one is 'prokaryotic lipid' which possesses C_{16} fatty acids at the *sn*-2 position, and both C_{16} and C_{18} acids at the *sn*-1

Table 4. Distribution of fatty acids at the *sn*-1 and *sn*-2 positions in the phospholipids from *P. tricornutum*

	Fatty acid composition (molar %)							
	PG				PC			
	A*		B†		A*		B†	
Fatty acid	*sn*-1	*sn*-2	*sn*-1	*sn*-2	*sn*-1	*sn*-2	*sn*-1	*sn*-2
14:0	1.8	0.2	1.8	0.2	6.8	0.8	6.3	1.3
16:0	10.6	30.4	12.2	28.8	6.5	13.1	7.0	12.6
16:1 (*n*-7)	24.7	6.5	31.2	0	19.5	8.1	20.0	7.6
16:1 (*n*-13)t	12.2	40.6	0	52.8	0	0	0	0
16:2	0	0	0	0	0.6	2.4	0.3	2.7
16:3	0	0	0	0	0.3	1.3	0.4	1.2
18:1	0	12.0	8.4	3.8	1.6	17.0	3.6	15.0
18:2	0	3.6	0.7	2.9	4.1	29.9	3.1	30.9
18:3	0	0	0	0	0.1	4.1	0.5	3.9
20:3	0.8	1.0	0	1.8	3.5	7.7	2.1	9.1
20:4 (*n*-6)	0	0	0	0	4.3	1.3	3.4	2.2
20:4 (*n*-3)	0	0	0	0	3.8	1.2	3.1	1.9
20:5	49.9	4.4	45.3	9.1	48.9	12.9	50.2	11.6

*Determined by *Rhizopus* lipase hydrolysis.
†Determined by phospholipase A_2 hydrolysis.

Table 5. Four types of *P. tricornutum* lipids

Group 1	Group2	Group 3	Group 4
X—C-20, C-16 X—C-16	X—C-14 X—C-16	X—C-20, C-16 X—C-18	X—C-20, C-16 X—C-20
MGDG	SQDQ	PC	PC
DGDG			
SQDG		MGDG*	MGDG*
PG		DGDG*	DGDG*
PC		SQDG*	
		PG*	

*Small amount.

position of glycerolipids. The other type is 'eukaryotic lipid' which possesses C_{18} fatty acids at the *sn*-2 and both C_{16} and C_{18} acids at the *sn*-1 position. In *P. tricornutum*, groups 1 and 2 of the lipids (Table 5) are the prokaryotic type in that they possess C_{16} acids at the *sn*-2 position. On the other hand, the lipids of group 3 are the eukaryotic type, because they have C_{18} acids at the *sn*-2 position. Group 4 lipid which possesses C_{20} fatty acids at the *sn*-2 position is also thought to be a eukaryotic type, provided that these acids are replaced by C_{18} acids in the eukaryotic lipid.

C_{20} fatty acids are located at the *sn*-2 position of MGDG from *Ginkgo*, *Pteridium*, *Equisetum* and *Catharinea* [10], in contrast to *P. tricornutum* in which 20:5 was located at the *sn*-1 position of most lipids including MGDG.

In 16:3 plants such as spinach and tobacco, PC was composed of the eukaryotic lipid, but PG of the prokaryotic lipid [11]. MGDG, DGDG and SQDG were composed of both the prokaryotic and eukaryotic lipids in 16:3 plants. In 18:3 plants such as wheat and cucumber, PG was composed of the prokaryotic lipid, but PC, MGDG, DGDG and SQDG were composed of the eukaryotic lipid [11]. On the other hand, in the diatom *P. tricornutum*, the lipid types are different from those in higher plants; PG, MGDG, DGDG and SQDG were mainly the prokaryotic types, whereas PC was composed of both prokaryotic lipid and the lipid corresponding to the eukaryotic lipid. In higher plants, the prokaryotic and eukaryotic lipids are synthesized by the prokaryotic and eukaryotic pathways, respectively [12]. In the diatom, the relation between the biosynthetic pathway and the types of lipids may be more complicated, because there exists the chain elongation reaction from C_{18} to C_{20} acid in the synthesis of 20:5.

EXPERIMENTAL

Culture conditions. P. tricornutum cells were grown at 20° with aeration in a 2N3P medium [13] under a 16 hr light (6000 lux) and 8 hr dark cycle.

Lipid analysis. Cells at lag phase (0.5×10^5 cells/ml), at logarithmic phase (5.0×10^6 cells/ml), and at early stationary phase (2.5×10^7 cells/ml), respectively, were collected by centrifugation and washed with sea H_2O. Lipids were extd from the cells by the procedure of ref. [14].

The positional distribution of fatty acids in the lipids was determined using *Rhizopus delemar* lipase (Seikagaku Kogyo, Tokyo) for both glycolipids and phospholipids according to ref. [15] and using phospholipase A_2 for phospholipids according to ref. [16]. The lyophilized powder (9.6 mg) of the snake venom from *Trimeresurus flavoviridis* was incubated with 5 ml of 50 mM NaOAc buffer, pH 5.4, at 100° for 2 min. After the centrifugation at 2500 rpm for 5 min, the supernatant was used as phospholipase A_2.

Fatty acids such as 14:0, 16:0, 18:0, 18:1, 18:2, 18:3, 20:4 (*n*-6) and 20:5 were identified by comparison of their R_t and MS with those of authentic samples. The positions of the double

bonds in 20:5 were found to be 5, 8, 11, 14, 17 by the deuteriodiimide reduction method [17]. 16:1 (*n*-7) was identified by the OsO_4 oxidation method [18] and *trans*-16:1 (*n*-13) by comparison of its R_t with that of *trans*-16:1 (*n*-13) from spinach PG. Fatty acids such as 16:2, 16:3, 16:4, 20:2, 20:3 and 20:4 (*n*-3) [19] were determined by GC Rt and GC/MS. Fatty acid composition was determined by FID-GC of the corresponding Me esters; for quantitative determination 22:0 was used as int. std.

GC and GC-MS. Fatty acid Me esters were analysed on a 1.5 m × 2 mm glass column containing 5% Silar 10C, 5% Thermon 3000 or 5% Shinchrom E-71. The column temp was 190° (Silar 10C), 210° (Thermon 3000) and 230° (Shinchrom E-71), with N_2 as carrier at a flow rate of 30 ml/min. MS were recorded with an ionizing current of 60 μA, an electron-accelerating voltage of 70 eV and an ion source temp of 250°.

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