Phytochemistry, Vol. 28, No. 3, pp. 805–810, 1989.
Printed in Great Britain.

0031-9422/89 $3.00+0.00

POSITIONAL DISTRIBUTION OF FATTY ACIDS IN GALACTOLIPIDS OF ALGAE

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(*Received in revised form* 9 *September* 1988)

Key Word Index—Brown algae; green alga; red algae; Rhodophyta; Phacophyta; Chlorophyta; fatty acid composition; galactolipids; polyunsaturated fatty acids; positional distribution.

Abstract—Fatty acid composition of both total lipids and galactolipids were determined for thalli from *Gloiopeltis complanata, Grateloupia filicina, Gymnogongrus flabelliformis,* (Rhodophyta), *Ishige okamurai, Padina arborescens, Sargassum ringgoldianum* (Phaeophyta) and *Monostroma nitidum* (Chlorophyta); the positional distribution of fatty acids in galactolipids of these algae was further analysed. C_{16} fatty acids were distributed at both the *sn*-1 and *sn*-2 positions of galactolipids from the red and brown algae, but mainly located at the *sn*-2 position of those from the green alga. In C_{18} fatty acids from brown algae, 18:1 and 18:2 were distributed only at the *sn*-2 position, with 18:3 and 18:4 at both the *sn*-1 and *sn*-2 positions. In the green alga, all C_{18} fatty acids were predominantly located at the *sn*-1 position of galactolipids. In red and brown algae, C_{20} fatty acids were exclusively located at the *sn*-1 position of galactolipids, but exceptionally at both the *sn*-1 and *sn*-2 positions in monogalactosyl diacylglycerols from red algae, C_{20} fatty acids being found more at the *sn*-1 than the *sn*-2 position.

INTRODUCTION

Although the major fatty acids of many marine algae consist of polyunsaturated C_{18} and C_{20} fatty acids [1], little is known about their biosynthesis. In higher plants the paths for lipid synthesis are related to the lipid molecular species; the prokaryotic lipid which possesses C_{18} or C_{16} fatty acids at the *sn*-1 position and C_{16} fatty acid at the *sn*-2 position is synthesized by the prokaryotic (chloroplast) pathway, and the eukaryotic lipid which possesses C_{18} or C_{16} fatty acids at the *sn*-1 position and C_{18} fatty acids at the *sn*-2 position is synthesized by the eukaryotic (cytoplasmic) pathway [2]. In the synthesis of prokaryotic monogalactosyl diacylglycerols (MGDG), 1-oleoyl-2-palmitoyl-phosphatidic acid synthesized in the chloroplast is metabolized to the corresponding MGDG and successively desaturated to 1-linolenoyl-2-hexadecatrienoyl-MGDG in the chloroplast. On the other hand, in the synthesis of eukaryotic MGDG, dioleoyl phosphatidylcholine synthesized in the endoplasmic reticulum is desaturated to dilinoleoyl phosphatidylcholine, the diacyl moiety of which is in turn transferred to the chloroplast from the endoplasmic reticulum to form dilinoleoyl-MGDG and desaturated to dilinolenoyl-MGDG in the chloroplast [2]. Thus, the molecular species of galactolipids in the chloroplast provides evidence for the type of pathway by which their lipids are synthesized. In galactolipids from the marine diatom *Phaeodactylum tricornutum*, C_{20} fatty acids are exclusively located at the *sn*-1 position, but C_{16} fatty acids are located at both *sn*-1 and *sn*-2 position. Galactolipids of this diatom contain little C_{18} acid where it is concentrated at the *sn*-2 position of phosphatidylcholine.

In this paper, the positional distribution of fatty acids in galactolipids from some red, brown and green algae is examined in order to gain some information about the possible biosynthesis of polyunsaturated C_{18} and C_{20} fatty acids contained in algal galactolipids.

RESULTS

Red algae

The fatty acid composition of the three red algae is shown in Table 1. 14:0, 16:0, 18:1 and 20:5 were the major fatty acids of the three algae. Also, 20:4 (*n*-6) was the major fatty acid in *Gloiopeltis complanata* and *Grateloupia filicina.* The composition and positional distribution of fatty acids in MGDG from the three red algae *G. complanata, G. filicina* and *Gymnogongrus flabelliformis* are shown in Table 2. The major fatty acids of MGDG were 16:0, 18:1 and 20:5 in the three algae and also 20:4 (*n*-6) in the former two. In MGDG from the three red algae, 16:0, 20:4 (*n*-6) and 20:5 were distributed in both the *sn*-1 and *sn*-2 positions, but most of the 18:1 and 18:2 were only in the *sn*-2 position. The composition and positional distribution of these fatty acids in digalactosyl diacylglycerols (DGDG) of the three red algae are the same as that in MGDG of these algae except for 20:5, (Table 3). 16:0, 18:1 and 20:5 were the major fatty acids of DGDG from the three red algae. 16:0 and 20:4 (*n*-6) were located at both positions and 18:1 and 18:2 were at the *sn*-2 position, but most of the 20:5 was restricted to the *sn*-1 position.

Brown algae

The fatty acid composition of the three brown algae is shown in Table 4. 16:0, 18:1, 20:4(*n*-6) and 20:5 were the major fatty acids of *Ishige okamurai, Padina arborescens*

Table 1. Fatty acid composition of total lipids from three Rhodophyta

Fatty acid	Fatty acid composition (molar %)		
	G. complanata	*G. filicina*	*G. flabelliformis*
14:0	8.5	13.8	9.4
16:0	43.2	50.8	45.0
16:1	0	0.4	0
16:2	0.8	0.2	1.2
16:3	0.5	0.3	0.5
18:0	1.0	1.0	1.9
18:1	11.1	11.2	18.0
18:2	0.8	1.0	3.0
18:3(*n*-6)	0.2	0.3	0.4
18:3(*n*-3)	0.5	0.1	0.6
18:4	0.5	0.2	0.1
20:3	0	0.7	0
20:4(*n*-6)	16.9	9.3	3.9
20:5	16.0	10.7	16.0

Table 2. Composition and positional distribution of fatty acids in monogalactosyl diacylglycerols from three Rhodophyta

Fatty acid	Fatty acid composition (molar %)								
	G. complanata			*G. filicina*			*G. flabelliformis*		
	Total	*sn*-1	*sn*-2	Total	*sn*-1	*sn*-2	Total	*sn*-1	*sn*-2
14:0	1.3	2.3	0.3	1.3	1.4	1.2	3.8	5.3	2.3
16:0	43.9	42.5	45.3	45.0	39.2	50.8	34.7	40.8	28.6
16:1	1.7	3.2	0.2	0	0	0	3.5	1.2	5.8
16:2	1.8	2.8	0.8	0	0	0	5.3	6.6	4.0
16:3	1.4	0.8	2.0	0.3	0.2	0.4	3.4	0	9.7
18:0	1.3	1.5	1.1	1.7	1.5	1.9	2.2	0	6.1
18:1	8.6	3.1	14.1	15.5	7.8	23.2	11.4	0.3	22.5
18:2	0.5	0.3	0.7	0.9	0.4	1.4	1.5	0	3.0
18:3(*n*-6)	0.1	0.1	0.1	0	0	0	0.1	0.1	0.1
18:3(*n*-3)	0.1	0.2	0	0.1	0	0.2	0.9	0.8	1.0
18:4	1.0	1.0	1.0	0.1	0.1	0.1	0	0	0
20:2	0	0	0	0.3	0.3	0.3	0	0	0
20:3	0	0	0	0	0	0	0.5	0	1.1
20:4(*n*-6)	12.8	13.8	11.8	8.5	12.4	4.6	2.7	1.5	3.9
20:5	25.5	28.4	22.6	26.3	36.7	15.9	30.0	48.1	11.9

and *Sargassum ringgoldianum*. Furthermore, 18:2 was also the major fatty acid in *I. okamurai*, whereas 18:3(*n*-3) and 18:4 were the major fatty acids in *P. arborescens* and *S. ringgoldianum*. The composition and positional distribution of fatty acids in MGDG of the three brown algae are shown in Table 5. 16:0, 18:3(*n*-3) and 18:4 in the three algae, 18:1 in *P. arborescens*, 18:2 in *I. okamurai* and 20:5 in both *I. okamurai* and *S. ringgoldianum* were the major fatty acids of MGDG. In MGDG from the three brown algae, 18:1 and 18:2 were dominantly located in the *sn*-2 positions, whereas most of the 20:4(*n*-6) and 20:5 were in the *sn*-1 position. On the other hand, 16:0, 18:3(*n*-3) and 18:4 in MGDG were generally distributed in both the *sn*-1 and *sn*-2 positions. The composition and positional distribution of fatty acids in DGDG of the three brown algae are shown in Table 6. The major fatty acids of the DGDG were 16:0, 18:3(*n*-3), 18:4 and 20:5 in the three algae and 18:2 in *I. okamurai*. The positional distribution of fatty acids in DGDG was similar to that in the MGDG of these brown algae; the distribution of 18:1 and 18:2 was restricted to the *sn*-2 position, whereas 20:4(*n*-6) and 20:5 was dominant in the *sn*-1 position; 16:0, 18:3(*n*-3) and 18:4 in the DGDG were principally located in both positions.

Table 3. Composition and positional distribution of fatty acids in digalactoysl diacylglycerols from three Rhodophyta

	Fatty acid composition (molar %)								
	G. complanata			*G. filicina*			*G. flabelliformis*		
Fatty acid	Total	*sn*-1	*sn*-2	Total	*sn*-1	*sn*-2	Total	*sn*-1	*sn*-2
14:0	0.4	0	3.0	1.5	0	7.3	3.3	4.2	2.4
16:0	47.3	58.6	36.0	55.0	68.1	41.9	48.0	67.6	28.4
16:1	0	0	0	0	0	0	3.4	0.8	6.0
16:2	0.7	0.7	0.7	0	0	0	1.3	1.8	0.8
16:3	0.5	0.1	0.9	0.2	0.4	0	0.8	0.4	1.2
18:0	1.0	0	3.8	1.8	0	3.7	6.2	10.0	2.4
18:1	18.5	0	44.6	21.4	0	45.2	19.5	0	47.6
18:2	0.8	0	3.1	0.9	0	1.9	1.9	0	5.5
18:3(*n*-6)	0	0	0	0	0	0	0.1	0.1	0.1
18:3(*n*-3)	0	0	0	0	0	0	0.8	0.7	0.9
18:4	0.3	0.6	0	0	0	0	0	0	0
20:4(*n*-6)	7.8	9.4	6.2	4.2	8.4	0	1.9	1.0	2.8
20:5	22.7	43.7	1.7	15.0	30.0	0	12.7	23.5	1.9

Table 4. Fatty acid composition of total lipids from three Phaeophyta

	Fatty acid composition (molar %)		
Fatty acid	*I. okamurai*	*P. arborescens*	*S. ringgoldianum*
14:0	6.7	5.6	6.7
16:0	22.9	25.5	26.1
16:1	2.1	10.6	4.0
16:2	0.5	1.5	1.3
16:3	0.3	0.5	0.5
18:0	1.0	0	0
18:1	14.2	12.6	8.3
18:2	25.2	5.3	4.3
18:3(*n*-6)	0.9	0.3	0
18:3(*n*-3)	3.6	9.9	15.2
18:4	3.3	11.2	10.1
20:3	0.9	0.2	0
20:4(*n*-6)	10.8	9.8	15.6
20:4(*n*-3)	0	1.7	1.2
20:5	7.6	5.3	6.7

Green alga

The fatty acid composition of total lipids and the composition and positional distribution of fatty acids in MGDG and DGDG from the green alga, *Monostrama nitidum*, are shown in Table 7. The major fatty acids in the total lipids were 14:0, 16:0, 16:4, 18:3(*n*-3) and 18:4. Among them 16:4, 18:3(*n*-3) and 18:4 were the major fatty acids in MGDG and additionally 16:0 and 16:3 in DGDG. In MGDG of *M. nitidum*, 16:0, 16:1, 16:2, 16:3 and 16:4 were exclusively located in the *sn*-2 positions, whereas 18:1, 18:2, 18:3(*n*-3) and 18:4 were located mainly in the *sn*-1 position. The same distribution of fatty acids was observed in the DGDG of the green alga, except for 16:4 and 18:3(*n*-3) which occurred at both the *sn*-1 and *sn*-2 positions.

DISCUSSION

The major fatty acids of the MGDG and DGDG in the red algae examined herein were 16:0 and 20:5, in agreement with those of *Porphyra yezoensis* [4], but 20:4 was an additional major fatty acid in the red algae examined. Similarly in two of the brown algae *P. arborescens* and *S. ringgoldianum*, the major polyunsaturated acids of the MGDG and DGDG were 18:3(*n*-3), 18:4 and 20:5, consistent with those in *Fucus serratus* [5], but in *I.*

Table 5. Composition and positional distribution of fatty acids in monogalactosyl diacylglycerols from three Phaeophyta

Fatty acid	Fatty acid composition (molar %)								
	I. okamurai			*P. arborescens*			*S. ringgoldianum*		
	Total	*sn*-1	*sn*-2	Total	*sn*-1	*sn*-2	Total	*sn*-1	*sn*-2
14:0	1.5	2.0	1.0	1.2	2.3	0.1	2.3	3.6	1.0
16:0	11.3	15.9	6.7	13.1	23.9	2.3	11.5	14.4	8.6
16:1	1.5	1.6	1.4	6.0	8.0	4.0	2.1	1.6	2.6
16:2	0.6	0.6	0.6	0.8	1.2	0.4	0.4	0.4	0.4
16:3	0.2	0.2	0.2	0.6	0.7	0.5	0	0	0
18:0	1.7	0.3	3.1	0.6	0.7	0.5	0.2	0	2.1
18:1	5.5	0	11.8	12.4	0	30.7	7.0	0	15.4
18:2	21.9	5.4	38.4	5.2	2.6	7.8	5.2	1.8	8.6
18:3(*n*-6)	3.1	1.9	4.3	1.2	0.6	1.8	0.3	0.4	0.2
18:3(*n*-3)	8.1	9.7	6.5	17.1	23.8	10.4	26.0	37.6	14.4
18:4	18.7	18.5	18.9	32.1	25.8	38.4	28.5	16.0	41.0
20:3	0.4	0.8	0	0.2	0.2	0.2	0	0	0
20:4(*n*-6)	6.8	10.4	3.2	2.3	3.4	1.2	4.0	5.6	2.4
20:4(*n*-3)	1.1	0.5	1.7	1.6	1.2	1.9	1.7	2.5	0.9
20:5	17.6	33.0	2.2	5.6	10.5	0.7	10.6	18.9	2.3

Table 6. Composition and positional distribution of fatty acids in digalactosyl diacylglycerols from three Phaeophyta

Fatty acid	Fatty acid composition (molar %)								
	I. okamurai			*P. arborescens*			*S. ringgoldianum*		
	Total	*sn*-1	*sn*-2	Total	*sn*-1	*sn*-2	Total	*sn*-1	*sn*-2
14:0	1.5	1.9	1.1	0.8	1.2	0.4	1.1	0.6	1.6
16:0	10.7	10.8	10.6	13.3	14.8	11.8	6.8	0.9	12.7
16:1	1.8	1.5	2.1	6.3	1.8	10.8	1.8	0.5	3.1
16:2	0.4	0.3	0.5	0.5	0.4	0.6	0.3	0	0.7
16:3	0.1	0	0.3	0.7	0.6	0.8	0.4	0.5	0.3
18:0	1.3	0	4.9	1.8	2.8	0.8	0.3	0	2.7
18:1	4.2	0	12.9	3.8	0	11.3	2.1	0	5.6
18:2	17.4	1.7	33.1	4.2	0	10.2	2.6	0	6.9
18:3(*n*-6)	2.5	2.5	2.5	0.6	0.8	0.4	0.1	0.2	0
18:3(*n*-3)	9.2	6.5	11.9	13.3	3.4	23.2	25.2	14.8	35.6
18:4	14.0	12.6	15.4	21.2	16.3	26.1	27.4	31.9	22.9
20:3	0	0	0	0.2	0.4	0	0	0	0
20:4(*n*-6)	6.9	11.9	1.9	4.6	7.4	1.8	7.2	11.0	3.4
20:4(*n*-3)	0	0	0	0.8	1.3	0.3	0	0	0
20:5	30.0	57.2	2.8	27.9	54.3	1.5	24.7	44.9	4.5

okamurai 18:2 was also the major polyunsaturated fatty acid. The major fatty acids of the MGDG and DGDG in *M. nitidum* were polyunsaturated acids such as 16:3, 16:4, 18:3 and 18:4 (Table 7), similar to those in other green algae [6].

The high amounts of 20:5 and 20:4 was characteristic of the fatty acid composition in the red and brown algae (Tables 1 and 4) [4, 5], and both polyunsaturated fatty acids were distributed in not only galactolipids, but also phospholipids (data not shown). In the diatom *P. tricornutum* containing C_{20} polyunsaturated fatty acids, the concentration of 20:5 was high, but that of 20:4 was extremely low [3]. Percentages of 20:5 in the MGDG and DGDG from this alga were 34.0 and 18.7%, respectively [3]. A relatively concentrated localization of both 16:4 and 18:4 in MGDG was found in the green alga, *M. nitidum* (Table 7).

The positional distribution of fatty acids in galactolipids of the red, brown and green algae so far examined are summarized in Table 8. The occurrence of 20:5 at the

Table 7. Fatty acid composition of total lipids, and composition and positional distribution of fatty acids in mono- and digalactosyl diacylglycerols from *M. nitidum*

	Fatty acid composition (molar %)						
Fatty acid	Total lipid	MGDG Total	MGDG *sn*-1	MGDG *sn*-2	DGDG Total	DGDG *sn*-1	DGDG *sn*-2
14:0	9.5	0.3	0.2	0.4	1.4	2.5	0.3
16:0	28.8	3.2	0.5	5.9	12.7	1.8	23.6
16:1	0.3	0.6	0.2	1.0	0.8	0	2.1
16:2	0.1	0.7	0.3	1.1	1.4	0.6	2.2
16:3	1.5	2.7	0	6.1	12.6	3.2	22.0
16:4	9.8	37.7	0	80.3	11.3	12.3	10.3
18:0	1.6	1.9	3.8	0	2.1	0.3	3.9
18:1	4.2	0.9	1.3	0.5	1.3	0	4.5
18:2	5.1	1.5	2.8	0.2	3.1	4.8	1.4
18:3(*n*-3)	27.6	33.2	64.4	2.0	43.1	64.3	21.9
18:4	7.7	14.9	28.4	1.4	6.9	11.2	2.6
20:3	0.1	0	0	0	0	0	0
20:4(*n*-6)	1.0	0.6	0.7	0.5	1.6	2.3	0.9
20:4(*n*-3)	1.7	0.9	1.6	0.2	1.2	0.9	1.5
20:5	1.0	0.9	1.4	0.4	0.5	0	2.8

Table 8. Positional distribution of the major fatty acids from some algae of the Rhodophyta, Phaeophyta and Chlorophyta

	Positional distribution of fatty acids					
Fatty acid	MGDG I	MGDG II	MGDG I & II	DGDG I	DGDG II	DGDG I & II
16:0			Rh, Ph			Rh, Ph
16:3					Ch	
16:4		Ch				Ch
18:1		Rh, Ph			Rh, Ph	
18:2		Ph			Ph	
18:3(*n*-3)	Ch		Ph			Ph, Ch
18:4	Ch		Ph	Ch		Ph
20:4			Rh			
20:5	Ph		Rh	Rh, Ph		

I and II represent the *sn*-1 and *sn*-2 positions, respectively.
Rh, rhodophyta; Ph, phaeophyta; Ch, chlorophyta.
Data summarized from the results of Tables 2, 3, 5, 6 and 7.

sn-1 position of MGDG ranged over the brown algae (Tables 5 and 6), the marine diatom *P. tricornutum*, and a raphydophyte *Heterosigma akashiwo* (unpublished data), whereas the location of 20:5 at both the *sn*-1 and *sn*-2 positions of MGDG was seen in the red algae (Table 2) [4], suggesting a phylogenic response in the distribution of this acid in algae.

From the viewpoint of fatty acid synthesis and desaturation, the location of long chain fatty acids such as C_{16} and C_{18} at the *sn*-2 position, in contrast to that of very long chain fatty acids such as C_{20} at the *sn*-1 position suggest the occurrence of acyltransferases specific for chain length. In galactolipids containing unsaturated C_{18} acids, 18:1 and 18:2 occur only in the *sn*-2 position, but 18:3 and 18:4 are found in both the *sn*-1 and *sn*-2 positions. If 18:0 is desaturated to 18:1 by acyl carrier protein desaturase and 18:1 is further desaturated to 18:3 after acyl transfer to lipid as in higher plants, these results suggests that the 18:1-lipid desaturase is different from that of 18:2- and 18:3-lipid desaturases. Alternatively, if these polyunsaturated fatty acids are trans-acylated to lipids after their desaturation, these results point to different specificities of acyltransferase(s) between mono- and polyunsaturated fatty acids.

The occurrence of 16:3 and 16:4 at the *sn*-2 position and that of 18:3 and 18:4 at the *sn*-1 position in the

MGDG and DGDG of the green alga suggests that successive unsaturation of C_{18}/C_{16}-galactolipid in the green alga. Similarly, the occurrence of 20:5 at the *sn*-1 position and that of 18:2 at the *sn*-2 position in the MGDG and DGDG of the brown algae suggests that desaturation of 20:4/18:1-galactolipid to 20:5/18:2-galactolipid occurs in the brown algae.

EXPERIMENTAL

Plant material. All algae were collected in April, 1986 from the coast of Izu-Shimoda, Shizuoka Prefecture, Japan.

Lipid extract and analysis. Each alga was washed with sea water, frozen in liquid N_2 and ground in a pestle. Crude lipids were extd from the ground powder according to ref. [7] and lipid classes were isolated by TLC as described previously [3]. Identification of lipid classes on TLC were performed by parallel runs with authentic samples and by stains with reagents characterizing lipids [8]. The positional distribution of fatty acids in the lipids was determined according to ref. [9]. Fatty acids were identified by both GC and GC-MS as previously described [3]. The analyses of fatty acids and lipid classes were carried out in duplicate.

Acknowledgements—We are grateful to Professor Chihara, M. and Professor Yokohama, Y. for their help with the collection of algae.

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