Hydrobiologia **204/205**: 513–519, 1990.
S. C. Lindstrom and P. W. Gabrielson (eds), Thirteenth International Seaweed Symposium.

Content of arachidonic and eicosapentaenoic acids in polar lipids from *Gracilaria* (Gracilariales, Rhodophyta)

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Key words: fatty acids, *Gracilaria*, red algae, seaweed

Abstract

Fatty acid composition, especially the distribution of eicosapolyenoic acids in several species of *Gracilaria*, was analyzed in relation to their taxonomy. The species have been grouped into two types based on distribution of these polyenoic acids: Type I, which contains palmitic, oleic and arachidonic acids as the major components, and Type II, which contains eicosapentaenoic acid in addition to Type I fatty acids. Octadecapolyenoic acids were detected only in trace amounts in each Type. A similar remarkable difference also was observed in the fatty acid composition of lipid classes. The major component of eicosapolyenoic acids in Type I was arachidonic acid in all lipid classes. In Type II, eicosapentaenoic acid was the major component in monogalactosyl diacylglycerol, digalactosyl diacylglycerol, sulfoquinovosyl diacylglycerol and phosphatidylglycerol. Arachidonic and eicosapentaenoic acids were contained in large amounts in Type II phosphatidylcholine. Grouping of *Gracilaria* species into Type I and Type II is not entirely consistent with morphological and taxonomic features, but the difference in fatty acid composition is likely due to genetic rather than to environmental factors.

Introduction

Many red algae contain arachidonic (20:4ω6) and/or eicosapentaenoic acid (20:5ω3) as the major components of polyenoic acids, which are not observed in higher plants (Pohl & Zurheide, 1979). However, in the red alga *Gracilaria verrucosa* (Hudson) Papenfuss, there are some disagreements among the results so far reported on the content of these acids. Pohl *et al.* (1968) and Takagi *et al.* (1985) reported that 20:4ω6 amounted to 60% of the total fatty acids from this alga, while 20:5ω3 was only a few per cent. On the contrary, Hayashi *et al.* (1974) reported that the content of both 20:4ω6 and 20:5ω3 in the same alga was about 10% and 20%, respectively. Kaneniwa *et al.* (1987) obtained a similar result to that of Hayashi *et al.* (1974). We also have analyzed the fatty acid composition of the major lipid classes from *G. verrucosa*, and found that the major component of eicosapolyenoic acid was 20:4ω6 in all lipid classes (Araki *et al.*, 1986a), as in the results of Pohl *et al.* (1968) and Takagi *et al.* (1985).

Several unidentified species of *Gracilaria*, which were very similar to *G. verrucosa* in morphology except for having a reddish color, were collected from the coast near Tokyo. On prelimi-

nary analysis, we found that these specimens contained both 20:4ω6 and 20:5ω3 as the major components of eicosapolyenoic acids, as in the results of Hayashi *et al.* (1974) and Kaneniwa *et al.* (1987). Such a disagreement as seen in these reports can be claimed to be derived from changes in environmental condition or collecting season, but our preliminary result did not exclude the possibility that the variation in fatty acid composition may be a manifestation of genetic features of the species examined.

These facts led us to compare the patterns of fatty acid composition among various species of *Gracilaria* collected from different depths and in different seasons.

Materials and methods

Algal thalli for lipid extraction were collected from various locations near Tokyo (Table 1).

Extraction of lipids from thalli was performed using a mixture of chloroform/methanol (1:1, v/v) according to Bligh & Dyer (1959). The lipid extract was separated into individual lipid classes by chromatographic procedures as reported previously (Araki *et al.*, 1986b) and subjected to combined column chromatography with DEAE-Sepharose CL-6B and silicic acid, followed by thin-layer chromatography (TLC). Each lipid class was separated by TLC (Merk, 5721), scraped off the plate and methylated with 5% hydrochloric acid in methanol at 90 °C for 2 h. The resulting fatty acid methylesters were analyzed by gas–liquid chromatography (GLC, Shimadzu GC-9A) under conditions set out previously (Araki *et al.*, 1986b).

Results

Total fatty acid composition

The patterns of total fatty acid composition from *Gracilaria verrucosa*, *G. textorii* (Sur.) DeToni, *G. bursa-pastoris* (Gmelin) Silva and *G. chorda* Holmes were very similar to each other. The major fatty acids of these species were 16:0 and 20:4ω6, and especially the latter, which amounted to 50 to 60% of the total fatty acids (Table 2). On the other hand, the major components were 16:0 and 20:5ω3 in *G. gigas* Harvey and three 'unidentified' species (*Gracilaria* sp.) collected at Nagai, Enoshima and Tateyama (Table 2) and accounted for 30–40% of the total fatty acids. Arachidonic acid was the third major fatty acid in these species. All *Gracilaria* species investigated contained 5 to 10% of oleic acid, but octadecapolyenoic acids were present only in trace amounts.

Table 1. Species of *Gracilaria* used for lipid extraction.

Species	Date	Locality
Gracilaria verrucosa	May 12, 1987	Nagai, Kanagawa Prefecture
Gracilaria verrucosa	Jul. 11, 1987	Nagai, Kanagawa Prefecture
Gracilaria verrucosa	Dec. 5, 1987	Nagai, Kanagawa Prefecture
Gracilaria bursa-pastoris	Jun. 24, 1987	Nagai, Kanagawa Prefecture
Gracilaria textorii	Jun. 24, 1987	Nagai, Kanagawa Prefecture
Gracilaria chorda	Jan. 25, 1988	Tateyama, Chiba Prefecture
Gracilaria gigas	Apr. 5, 1988	Shimoda, Shizuoka Prefecture
Gracilaria sp.*	May 12, 1987	Nagai, Kanagawa Prefecture
Gracilaria sp.*	Jul. 28, 1987	Nagai, Kanagawa Prefecture
Gracilaria sp.*	Dec. 5, 1987	Nagai, Kanagawa Prefecture
Gracilaria sp.*	Jun. 24, 1987	Enoshima, Kanagawa Prefecture
Gracilaria sp.*	Jul. 13, 1987	Tateyama, Chiba Prefecture**

* Unidentified species that are very similar in morphology to *G. verrucosa*, but that have reddish thalii.
** Cystocarp-bearing specimen.

Table 2. Fatty acid composition (molar %) of different species of *Gracilaria*. tr, trace amount.

Species	16:0	18:1	18:3	20:3	20:4 ω6	20:5 ω3
Type I						
G. verrucosa (May 12)	31	5	1	3	54	2
G. bursa-pastoris	30	7	1	1	51	tr
G. textorii	27	5	1	tr	61	1
G. chorda	26	8	tr	1	55	1
Type II						
G. gigas	30	8	1	2	12	38
G. sp. (Nagai)	35	8	1	1	10	39
G. sp. (Enoshima)	33	10	1	tr	23	30
G. sp. (Tateyama)	35	8	1	2	18	30

Four *Gracilaria* species classified in the first group in Table 2 were remarkably different in the content of 20:4ω6 and 20:5ω3 from those in the second group. Thus, we call tentatively these two groups 'Type I and Type II', respectively, in this paper.

Lipid composition

Lipid contents, which were evaluated from fatty acid content of fresh thalli, ranged from 0.03 to 0.16% among the *Gracilaria* species tested (Table 3). Seven lipid classes were determined in each species: 1) three glycolipids, monogalactosyl diacylglycerol (MGDG), digalactosyl diacylglycerol (DGDG) and sulfoquinovosyl diacylglycerol (SQDG), 2) three phospholipids, phosphatidylglycerol (PG), phosphatidylcholine (PC) and phosphatidylethanolamine (PE), and 3) triacylglycerole (TG). MGDG, DGDG, SQDG and PC were major components, and they amounted nearly to 90% of the total lipid. PG, PE and TG were minor components (Table 3). There were no noticeable differences between Type I and Type II *Gracilaria* species in their patterns of lipid composition.

Table 3. Lipid content (g of fatty acid per 100 g fresh wt) and composition (molar %) of different species of *Gracilaria*.

Species	Lipid content	Lipid composition						
		MGDG	DGDG	SQDG	PG	PC	PE	TG
Type I								
G. verrucosa (May 12)	0.15	20.8	23.0	14.7	2.5	25.5	1.6	4.4
G. bursa-pastoris	0.06	27.2	15.0	26.9	2.6	24.1	1.4	3.3
G. textorii	0.16	23.5	17.9	17.6	2.2	31.5	0.3	5.2
G. chorda	0.03	31.2	18.3	18.1	2.5	26.4	tr	3.2
Type II								
G. gigas	0.08	20.4	11.8	18.1	4.7	22.7	0.6	4.8
G. sp. (Nagai)	0.14	27.5	17.9	21.3	3.0	18.3	1.6	2.8
G. sp. (Tateyama)	0.11	24.9	17.5	21.2	2.6	25.5	1.3	7.0

Abbreviations: MGDG, monogalactosyl diacylglycerol; DGDG, digalactosyl diacylglycerol; SQDG, sulfoquinovosyl diacylglycerol; PG, phosphatidyl glycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; TG, triacylglycerol.

Fatty acid composition of individual lipid classes

The fatty acid composition of MGDG, DGDG and SQDG in two selected species from each Type are shown in Table 4. MGDG contained polyenoic fatty acids in the highest level of the three glycolipids. DGDG and SQDG contained more saturated fatty acids than MGDG. However, a significant difference was found between the two types of *Gracilaria* species in the composition of their eicosapolyenoic acids; Type I species contained 20:4ω6, whereas Type II contained 20:5ω3 as the major polyenoic fatty acids.

Fatty acid composition of two phospholipids, PG and PC, and TG are shown in Table 5. *Trans* ω13-hexadecenoic acid (16:1*t*), which is contained exclusively in PG in many photosynthetic plants, also was detected in *Gracilaria* PG. As in the glycolipids (Table 4), the major eicosapolyenoic acid of PG was also 20:4ω6 in Type I and 20:5ω3 in Type II. In contrast, Type I contained

Table 4. Fatty acid composition (molar %) of monogalactosyl diacylglycerol (MGDG), digalactosyl diacylglycerol (DGDG) and sulfoquinovosyl diacylglycerol (SQDG) in the two types of *Gracilaria* species. –, not detected; tr, trace amount.

Fatty acid	Type I						Type II					
	G. verrucosa (May 12)			*G. chorda*			*G. gigas*			*G.* sp. (Nagai)		
	MGDG	DGDG	SQDG	MGDG	DGDG	SQDG	MGDG	DGDG	SQDG	MGDG	DGDG	SQDG
14:0	–	–	12	–	2	13	–	1	6	1	1	8
16:0	15	44	59	18	42	57	24	44	59	28	47	67
18:1	2	7	1	12	13	1	10	16	1	10	15	–
18:2	tr	1	tr	1	1	–	1	1	tr	1	1	–
18:3ω6	tr	tr	–	–	–	–	tr	tr	–	–	tr	–
18:3ω3	tr	tr	tr	tr	tr	–	–	–	–	–	–	tr
20:3	tr	tr	tr	1	1	–	1	1	–	1	1	–
20:4ω6	75	47	27	60	38	28	5	2	3	10	5	5
20:5ω3	3	1	tr	1	1	1	57	30	28	56	31	16

Table 5. Fatty acid composition (molar %) of phosphatidylglycerol (PG), phosphatidylcholine (PC) and triacylglycerol (TG) of two types of *Gracilaria* species. –, not detected; tr, trace amount.

Fatty acid	Type I						Type II					
	G. verrucosa (May 12)			*G. chorda*			*G. gigas*			*G.* sp. (Nagai)		
	PG	PC	TG	PG	PC	TG	PG	PC	TG	PG	PC	TG
14:0	–	1	5	–	–	–	–	–	2	tr	–	3
16:0	35	15	19	25	7	15	23	14	25	30	15	19
16:1	–	tr	17	–	tr	3	–	–	5	–	–	5
16:1*t*	11	–	–	22	–	–	26	–	–	18	–	–
18:1	8	7	6	2	5	11	3	4	20	5	6	8
18:2	1	1	1	tr	1	1	tr	1	2	1	1	1
18:3ω6	–	1	–	–	1	2	–	3	1	tr	2	1
18:3ω3	tr	1	–	2	1	–	tr	tr	tr	tr	–	tr
20:3	–	–	3	–	4	–	–	4	2	tr	5	2
20:4ω6	37	73	34	41	79	61	8	27	11	7	45	21
20:5ω3	3	1	4	2	1	3	34	44	27	38	23	24

Table 6. Fatty acid composition (molar %) of *Gracilaria verrucosa* (Type I) and *Gracilaria* sp. (Type II) collected in winter and summer at Nagai. Degree of desaturation estimated by the ratio of eicosapolyenoic to hexadecanoic acids.

Fatty acid	*G. verrucosa* (Type I)		*G.* sp. (Type II)	
	Dec. 5, 1987	July 11, 1987	Dec. 5, 1987	July 28, 1987
14:0	2	3	1	2
16:0	27	31	24	36
18:1	5	5	6	7
18:2	tr	tr	1	1
20:3	tr	tr	2	2
20:4ω6	62	54	12	14
20:5ω3	1	1	47	34
Degree of desaturation	2.3	1.7	2.5	1.4

only 20:4ω6 in PC, whereas both 20:4ω6 and 20:5ω3 were contained in Type II. This lipid class was highly desaturated as in MGDG.

Fatty acid composition in different seasons

The fatty acid compositions of *Gracilaria verrucosa* (Type I) and *Gracilaria* sp. (Type II) collected at Nagai in summer and winter were compared (Table 6). The average seawater temperature at the collection site was about 23 °C in summer (July) and about 15 °C in winter (Dec.).

The content of eicosapolyenoic acids in both types was higher in winter than in summer. Therefore, the desaturation degree, which is estimated by the ratio of eicosapolyenoic to hexadecanoic acids in both types, is necessarily higher in winter than in summer (Table 6). However, it appears that the characteristic pattern of the fatty acids in Type I and Type II species, in which the major eicosapolyenoic acid in the former is 20:4ω6 and that in the latter is 20:5ω3, is not affected by environmental factors such as seawater temperature in different seasons.

Discussion

Gracilaria verrucosa has been known to be distinct in its fatty acid composition in that it contains only 20:4ω6 as a polyenoic acid (Pohl *et al.*, 1968). The alga is different in this respect from many other red algae, which contain both 20:4ω6 and 20:5ω3 (Pohl & Zurheide; 1979, Araki *et al.*, 1986b). However, the same pattern of fatty acid composition as seen in *G. verrucosa* was found in other Type I species analyzed in the present work, while Type II species had a pattern similar to that of many other red algae (Pohl & Zurheide, 1979). Thus, the results of Pohl *et al.* (1968) and Takagi *et al.* (1985) agree with those of Type I species, and the results of Hayashi *et al.* (1974) and Kaneniwa *et al.* (1987) with Type II species.

The presence of two patterns among different *Gracilaria* species with respect to fatty acid composition implies the occurrence of different processes in the desaturation of fatty acids. Possibly the presence or absence of enzyme systems leading to the formation of 20:5ω3 may underlie the difference between Type I and Type II. If so, the question arises whether the difference is a genetic character of each species or simply derived from the influence of environmental factors surrounding the algal habitat.

Kaneniwa *et al.* (1987) thought that the variation in the fatty acid composition of *G. verrucosa* might be caused by different environmental conditions. In fact, it has been reported for various algae that the fatty acid composition is influenced by environmental factors such as temperature (Raison, 1980), light (Nichols, 1965) and nutrient

concentration in medium (Piorreck *et al.*, 1984). Many workers (Sato *et al.*, 1979; Kayama *et al.*, 1985; Lynch *et al.*, 1984a, 1984b) reported that a decrease in temperature induced more desaturation of algal fatty acids. Our present work showed that the amount of polyenoic fatty acids is greater in winter than in summer in both *G. verrucosa* (Type I) and *Gracilaria* sp. (Type II), while the characteristic pattern of the fatty acid composition in each type is preserved (Table 6). This fact suggests that the characteristic pattern of fatty acids in each type is not necessarily dependent on ambient temperature alone.

The effect of light intensity on fatty acid composition was studied by Constantopouls & Bloch (1967); they reported that an increase in light intensity increased the desaturation of fatty acids in algae. Increase in water depth results in the exponential decrease of light intensity and concomitant changes of light quality. *G. textorii* (Type I) and *G. gigas* (Type II) grow at similar water depths, while *G. verrucosa* (Type I) grows at a shallower depth than other species belonging to Type I. The characteristic fatty acid composition found in Type I and Type II species is thus apparently not a function of water depth. When the fatty acids of thalli of different ages of *G. verrucosa* (Type I) and *G. gigas* (Type II) were analyzed, we could confirm the characteristic pattern of fatty acid composition found in both species regardless of age (data not shown). Thus, it is reasonable to assume that the differences between Type I and Type II reflect genetic characteristics of the species.

Ohmi (1958) proposed classifying *G. chorda* as a *Gracilariopsis* according to Dawson's system (Dawson, 1949), because it possesses no nutritive filaments in its cystocarps. We collected cystocarp–bearing thalli of *Gracilaria* sp. (Type II) at Tateyama and no nutritive filaments were found (unpublished data). From this criterion, this *Gracilaria* sp. should be included in *Gracilariopsis*. On the other hand, nutritive filaments have been observed in *G. gigas*, another Type II species, and this alga was classified as a *Gracilaria* by Ohmi. In recent years, the information from morphological studies of spermatangia often has been applied to the systematics of Gracilariaceae. According to Yamamoto's system (1978), which is based on the differences in the morphology and development of male organs, *G. chorda* is classified as 'Chorda type' and *G. gigas* as 'Verrucosa type'. Fredericq & Hommersand (1989) confirmed that *Gracilariopsis* is distinct from *Gracilaria* at the generic level on the basis of their morphological studies on spermatangial development and post-fertilization events. Thus, our grouping of *Gracilaria* species, in which the species are classified as Type I and Type II according to their characteristic fatty acid composition, is not entirely compatible with current morphological and systematic thinking.

On the other hand, it has been known that properties of the sulfated polysaccharides of the cell wall of *Gracilaria* are different between tetrasporophytes and gametophytes (Hoyle, 1978; Shi *et al.*, 1984). Thus, it is possible that a difference in the fatty acid composition will also be found between these two generations.

The present results have shown that there are two types of species of *Gracilaria* that differ in their pattern of fatty acids. This difference may be related to a genetic character of the species. Moreover, it is of interest to note that the lipids from *Hypnea charoides* and *Chondrus ocellatus* show a similar fatty acid pattern to that observed in Type II species, although the data are not presented here. Therefore, the full significance of Type I and Type II species requires further detailed study.

Acknowledgements

We thank Professor Dr. Mitsuo Chihara, Tsukuba University, for identification of the *Gracilaria* species and for useful suggestions.

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