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Lipid and fatty acid composition in the red alga *Porphyra yezoensis*

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Lipids of *Porphyra yezoensis* thalli cultured at 13°C for 4 weeks were extracted with organic solvents. They were fractionated by column chromatography on DEAE-Sepharose CL-6B and on silicic acid and then separated by thin-layer chromatography. Monogalactosyl diacylglycerol, digalactosyl diacylglycerol, phosphatidylglycerol, sulfoquinovosyl diacylglycerol, phosphatidylcholine, phosphatidylethanolamine and triacylglycerol were identified as major lipid components.

Major fatty acid components of the lipid classes were palmitic and eicosapentaenoic acids, except for phosphatidylglycerol and phosphatidylethanolamine. Phosphatidylglycerol contained large proportions of *trans* ω13 hexadecenoic acid and a C_{20} monoenoic acid, and phosphatidylethanolamine contained C_{20} polyunsaturated acids which amounted to 85% of the total fatty acids. Both α- and γ-linolenic acids were detected. The γ-isomer was associated mainly with in phosphatidylcholine, phosphatidylethanolamine and triacylglycerol.

Key Index Words: *Fatty acid*; *lipid*; Porphyra yezoensis; *red alga*; *Rhodophyceae*.

The fatty acid composition of marine algae is remarkably different from that of higher plants in containing high levels of polyunsaturated fatty acids of 20 carbon atoms (POHL and ZURHEIDE 1979). In the red alga, *Porphyra*, eicosapentaenoic acid amounts to about 50% of total fatty acids (KAYAMA *et al.* 1983). Since this acid is one of the precursors of prostaglandins in animals (PIKE 1971), the lipids of the dried laver, "Hoshinori", which is a traditional foodstuff produced from *Porphyra* thalli in Japan, has high nutritional value.

There is, however, only limited information on the lipid and fatty acid composition of *Porphyra*. SATO (1971) separated the glycolipids from the thalli of *Porphyra tenera* and reported the occurrence of MGDG, DGDG and SQDG, and that the major fatty acids were palmitic and eicosapentaenoic acids. SAKAMOTO and ENOMOTO (1975,

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Abbreviations: MGDG, monogalactosyl diacylglycerol; DGDG, digalactosyl diacylglycerol; PG, phosphatidylglycerol; SQDG, sulfoquinovosyl diacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PA, phosphatidic acid; SPM, sphingomyelin; TG, triacylglycerol. In fatty acid shorthand such as 16:0, 20:5 etc, the colon separates figures denoting the number of carbon atoms and the number of double bonds respectively in the molecule. 16:1 *t*, *trans* ω13 hexadecenoic acid; 18:2ω6, linoleic acid; 18:3ω3, α-linolenic acid; 18:3ω6, γ-linolenic acid; 20:4ω6, arachidonic acid; 20:5ω3, eicosapentaenoic acid.

1976a, b) also studied the lipids from "Hoshi-nori". In contrast to the results of Sato, they found that the constituent sugars of the diglycosyldiacylglycerol were galactose and mannose, and that 16:0, 20:3 and 22:6 acids were the major fatty acids. ANDO and KANEDA (1968) examined the phospholipids from "Hoshi-nori" and identified PC, PE, PS, PI, PA and SPM. However, they described nothing on the fatty acid composition of these phospholipids. Moreover, they did not detect PG, which is widely distributed in photosynthetic plants (HARWOOD, 1980) and which has been reported to occur in *Porphyra yezoensis* by SAKAMOTO and ENOMOTO (1976b). It seems worthy of note to elucidate this apparent inconsistency, not only from the biological standpoint, but also for the development of the more rational methods of processing and storing the "Hoshi-nori".

In the present paper, we will describe the fatty acid composition of lipids extracted from cultured *Porphyra* thalli, and discuss their differences from the results of earlier workers.

Materials and Methods

Culture of Porphyra yezoensis. The germlings from conchospores were inoculated into a culture flask containing one litre of artificial seawater (SUTO's ASP 6 modified medium) and grown at 18°C, under aeration and with a light intensity of 10,000 lux. After three weeks, the thalli were transferred into a 10-litre flask, and grown for further 4 weeks at 13°C under the same light intensity, with weekly renewing of the medium. The thalli, which had grown up to 8-10 cm in length, were then harvested for lipid extraction. The culture was illuminated by a hallogen lamp (Toshiba), and the light regime was 10L-14D a day. The culture medium was maintained in a range of pH 8.0 to 8.5.

Extraction, Separation and Identification of Lipids. The lipids were extracted from the thalli with chloroform/methanol (1: 2, v/v) according to the procedures of BLIGH and DYER (1959). The extract was concentrated under reduced pressure, dissolved in a small volume of chloroform/methanol (1: 4, v/v), and then fractionated by the method of MURATA *et al.* (1982) as follows. The lipid solution was applied to a DEAE-Sepharose CL-6B column (50 mm×20 mm, internal diameter) and eluted with 100 ml of chloroform/methanol (1: 4, v/v). This eluate (fraction A) was stored in a refrigerator until use. The column was then successively eluted with 100 ml of acetic acid (fraction 4) and chloroform/methanol (1: 4, v/v) containing 0.2% (w/v) ammonium acetate (fraction 5).

The fraction A was concentrated under reduced pressure, and dissolved in a small volume of chloroform. It was then applied to a column (50 mm×20 mm, internal diameter) of silicic acid (Iatrobeads 6RS-8060), and was successively eluted with 25 ml of chloroform/acetone (4: 1, v/v, fraction 1), 100 ml of acetone (fraction 2) and 50 ml of methanol (fraction 3).

The lipids in each fraction were further separated on precoated silica gel plates (Merk, 5721), using chloroform/methanol/water (70: 21: 3, v/v) as the developing solvent of TLC for fractions 1, 2 and 3 and chloroform/acetone/methanol/acetic acid/water (50: 20: 10: 15: 5, v/v) for fraction 4 and 5. The lipids separated on the plates were identified by comparing their Rf values with those of standard lipids from spinach leaves, and with visualizing reagents.

Analysis and Determination of the Fatty Acids and Sugars. The lipids separated on the TLC plate were located by a fluorescent dye, primuline. They were scraped off the TLC plate and treated with 5% hydrochloric acid in methanol at 90°C for 2 h. The resulting fatty acid methyl esters were extracted with *n*-hexane and analysed in a gasliquid chromatograph (Shimadzu GC-9A) equipped with a hydrogen flame ionization detector. The GLC column was a 2 m×3 mm glass column packed with 5% Thermon 3000 on Shimalite W, AW-DMCS (201D). Column temperature was 210°C and N_2 carrier gas

flow was 60 ml/min. Pentadecanoic acid was used as an internal standard.

The identification of individual fatty acids was carried out by gas chromatography-mass spectrometry; fatty acid methyl esters were applied to a glass column (2 m×2.6 mm) containing 5% Shinchrome E71 on Shimalite AW(80-100 mesh) and were chromatographed at 180°C with helium as a carrier gas at a flow rate of 30 ml/min. Mass spectra were taken every 3.0 sec with a GCMS-QP 1000 spectrometer (Shimadzu), with an electron-accelerating voltage of 70 eV and an ion source temperature of 250°C.

The sugar component of glycolipids were analysed as follows. Methylglycosides, recovered from the methanol phase after extracting the fatty acid methyl esters of glycolipids, were trimethylsilylated with a mixture of hexamethyldisilazane and trimethylchlorosilane in pyridine (SWEELEY and WALKER 1964). The trimethylsilylated sugars were analysed and identified by GLC using silicone SE-30 as a liquid phase at column temperature of 175°C. Mannitol was used as internal standard.

Results

The lipids extracted from *Porphyra* thalli were separated by the combined procedures of column and thin-layer chromatography. MGDG, DGDG, PG, SQDG, PC, PE and TG were identified as the major lipid classes (Table 1).

When the fraction 2 (glycolipid fraction)

Table 1. Lipid composition of *Porphyra yezoensis*.

Lipids	Molar %
MGDG	27.2
DGDG	24.5
PG	19.0
SQDG	10.6
PC	12.2
PE	3.2
TG	3.4

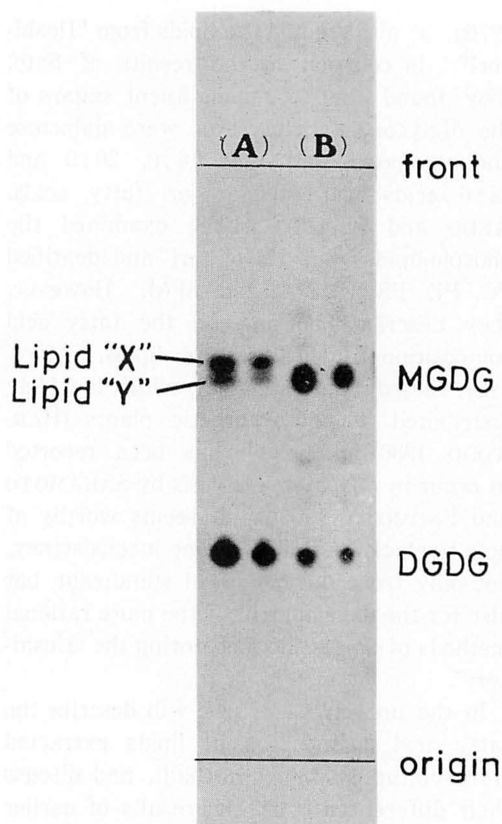

Fig. 1. TLC of fraction 2 from *Porphyra yezoensis* (A). Developmental solvent, chloroform/methanol/water (70 : 21 : 3, v/v); visualized reagent, anthron reagent. The glycolipids from spinach leaves are shown for comparison (B).

was developed on the TLC plate, double spots appeared close to the position equivalent to MGDG (Fig. 1). The Rf of the lower spot was the same as that of spinach MGDG. These spots were positive with the anthrone reagent (YAMAKAWA *et al.* 1960), suggesting that both are glycolipids. Then, in order to examine whether one of the two spots was caused by a glycolipid other than MGDG, the sugar moieties and fatty acid compositions of the lipids of the two spots were investigated.

After TLC of fraction 2, the upper-spot (lipid "X") and the lower spot (lipid "Y") were separately scraped off from the plate, and their sugar and fatty acid compositions were analyzed. The results were shown in Table 2. The constituent ratio of sugar to

Table 2. Analysis of galactose and fatty acids of monoglycolipids and DGDG from *Porphyra yezoensis*.

	Lipid "X"	Lipid "Y"	DGDG
Galactose (nano mole)	69	63	430
Fatty acids (nano mole)	134	105	418
Galactose / Fatty acid	0.51	0.60	1.03

fatty acids in both lipids "X" and "Y" was close to 1:2, and the sugar component was only galactose. Thus, both lipids are identified as MGDG. However, in lipid "X" the 20:5 acid amounted to about 90% of total fatty acids, while in lipid "Y" both the 20:5 and 16:0 acids each comprised about 40% of the total (Table 3). The sugar component of DGDG was also analyzed and identified as galactose.

The fatty acid composition of the lipid classes from *Porphyra* thalli are shown in Table 4. The fatty acids comprised about

Table 3. Fatty acid composition of the kinds of monoglycolipids from *Porphyra yezoensis*.

	Molar %	
	Lipid "X"	Lipid "Y"
14 : 0	1	4
16 : 0	2	41
18 : 0	tr	2
18 : 1	1	8
18 : 2	tr	4
20 : 3	1	4
20 : 4	2	2
20 : 5	93	32

3% of the dry weight of the thalli, while the total lipid content of "Hoshi-nori" was reported to be 2% of dry weight (RESOURCES COUNCIL, 1982).

In agreement with the results of earlier workers (SATO 1971, KAYAMA *et al.* 1983), the major components of the total fatty acids were palmitic and eicosapentaenoic acids, but the content of C_{16} and C_{18} polyunsaturated

Table 4. Fatty acid composition of the lipids from *Porphyra yezoensis*.

	Molar %							
	Total	MGDG	DGDG	PG	SQDG	PC	PE	TG
14 : 0	0.4	1.2	0.1	0.1	0.1	—	0.5	0.4
16 : 0	25.6	12.8	38.1	30.8	49.6	9.5	1.5	13.1
16 : 1 t	2.9	—	0.0	15.1	0.0	—	0.4	0.0
18 : 0	0.7	—	—	0.6	—	1.0	0.8	
18 : 1	3.6	3.6	6.7	1.0	0.5	4.6	1.8	10.3
18 : 2	2.0	1.4	4.2	0.1	0.1	4.2	1.1	6.6
18 : 3ω6	0.6	0.6	0.05	0.0	0.0	3.0	2.1	1.3
18 : 3ω3	0.3	0.2	0.3	0.3	0.0	0.6	0.0	0.3
18 : 4	0.5	0.1	0.1	0.1	0.2	2.5	1.4	1.1
20 : 1	3.5	0.7	0.9	14.7	0.2	0.8	1.3	4.0
20 : 2	1.4	0.7	0.8	4.6	0.0	0.3	0.3	2.2
20 : 3	2.2	3.0	1.9	0.0	0.0	2.8	7.7	7.8
20 : 4ω6	2.4	1.8	0.6	1.0	0.7	5.5	15.9	11.0
20 : 5ω3	53.2	73.8	45.7	29.9	48.4	64.6	60.0	41.2
22 : 1	0.3	0.0	0.0	1.2	0.0	0.2	0.0	0.3
Unknown	0.6	0.2	0.5	0.6	0.5	0.3	5.0	0.7

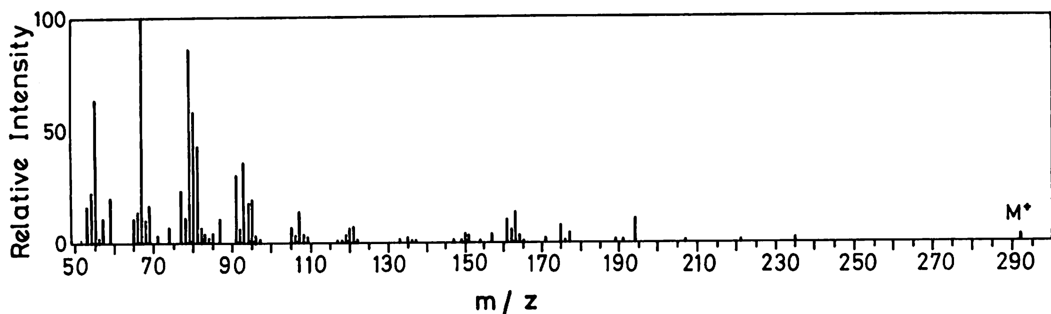

Fig. 2. Mass spectrum of methyl γ-linolenate from *Porphyra yezoensis*.

acids was relatively low.

The major fatty acid components of MGDG, DGDG and SQDG from *Porphyra* thalli were 16:0 and 20:5 which together comprised over 80% of the total fatty acids. On the other hand, PC and PE contained higher levels of C_{20} polyunsaturated fatty acids (20:3, 20:4 and 20:5) than the other lipids. PG differed from the other lipids in its high content of 16:1, 20:1 and 22:1 acids. A comparison of the retention time of the 16:1 acid from *Porphyra* PG with that of fish-oil (San omega), which contained only a *cis*-ω7-isomer, and that of spinach PG which contained only a *trans*-ω13-isomer suggested that the 16:1 acid of PG from *Porphyra* was identical with *trans*-ω13-16:1 acid of PG from spinach leaf.

γ-Linolenic acid, whose existence has not yet been reported in *Porphyra*, was also detected as a minor component. When the retention time of α- and γ-linolenate from *Porphyra* thalli was compared with that of the standard acids on two different GLC columns, Thermon 3000 and Shinchrome E 71, the values were completely consistent with those of the standards. Further, GC-MS spectra of both isomers from *Porphyra* also were identical with those of the standards (Fig. 2). These results lead us to conclude that the *Porphyra* thalli contain both α- and γ-linolenate. In *Porphyra*, the γ-isomer was found mainly in PC, PE and TG. However, MGDG and PC contained both isomers.

Discussion

In the present study we determined the major lipid and fatty acid compositions of the artificially grown *Porphyra* thalli, (see Tables 1 and 4). ANDO and KANEDA (1968) studied the phospholipids from commercial "Hoshi-nori", and reported the occurrence of PC, PE, PS, PI and PA. However, we could not detect the latter three components. Whether these lipids exist in *Porphyra* requires further studies, since they are only minor components of plant lipids (KATES 1970). In contrast to ANDO and KANEDA (1968) we found a significant content of PG, which is a constituent lipid of chloroplast thylakoids (HARWOOD 1980) and widely distributed in all the eukaryotic algae (JAMIESON and REID 1972).

TLC of the glycolipids from *P. yezoensis*, revealed a double spot close to the position equivalent to MGDG. SATO and MURATA (1982) demonstrated the occurrence of monoglucosyl diacylglycerol in the blue-green alga, *Anabaena variabilis*, and reported that the glucolipid migrated slightly faster than MGDG in TLC. On the other hand, SAKAMOTO and ENOMOTO (1976a) studied the glycolipids from the dried laver, "Hoshi-nori", and reported that mannose was one of the constituent sugars of glycolipids. The present study found that both components of the double spot contained only MGDG. However, the two components differed in fatty acid composition; while 20:5 comprised about 90% of the total fatty acid in the upper spot, both 20:5 and 16:0 acids amounted to

about 40% in the lower one. These results suggest that the upper component of MGDG consisted mainly of the molecular species 20:5/20:5, whereas the lower contained predominantly 20:5/16:0. Similar results were also obtained with MGDG from *Gracilaria verrucosa*, which contained a higher content of 20:4 than 20:5 acid (unpublished data).

Preliminary analyses of the total fatty acids of glycerolipids from another red alga, *G. verrucosa* also showed that γ-linolenate was more abundant than α-linolenate (data not shown). Most but not all red algae contained higher amounts of the α-isomer than the γ-isomer (POHL and ZURHEIDE 1979). However, further analyses are necessary before a general conclusion about the distribution of the linolenate isomers in lipids of the red algae can be drawn.

The red algae constitute a most primitive group of eukaryotic algae, and are systematically placed between the blue-green algae of prokaryotes and the cryptomonads of eukaryotes. Common and characteristic features of the three algal groups are the accessory pigments of photosynthesis, phycoerythrin and phycocyanin, and thylakoid ultrastructures that are much simpler than those of the other algal groups such as green and brown algae. However, as far as the lipid and fatty acid composition of the three groups are concerned, *Porphyra* is more closely related to the cryptomonads than to the blue-green algae since it contains as major phospholipids, PC and PE and *trans*-ω13-hexadecenoic acid as a major fatty acid of PG, all of which are lacking in the blue-green algae (KATES, 1970). However, the fatty acid composition of cryptomonads considerably differs from that of *Porphyra*. BEACH *et al.* (1970) showed that cryptomonads contain 18:3 and 18:4 acids as the major fatty acid components, whereas they are rather minor components in *Porphyra*. These authors further reported that 18:4 acid accounted for about 70% of the total fatty acids in MGDG and DGDG from *Cryptomonas* sp. WH.

In *Porphyridium cruentum*, the unicellular form of Bangiales in Rhodophyta, the total fatty acid composition is similar to *Porphyra* in that C_{18} unsaturated fatty acids are minor, and 16:0 and 20:5 acids are major components (NICHOLS and APPLEBY 1969). However, this alga contains a larger amount of 20: 4 than 20: 5 acid, especially in PC (NICHOLS and APPLEBY 1969). *Porphyra* is unique in having a particularly high content of 20:5 acid in all its lipid classes.

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荒木　繁*・桜井武麿*・小俣達男,1)・川口昭彦**・村田紀夫***：スサビノリの脂質と脂肪酸組成**

室内培養で得られたスサビノリ藻体から，monogalactosyl diacylglycerol, digalactosyl diacylglycerol, phosphatidylglycerol, sulfoquinovosyl diacylglycerol, phosphatidylcholine, phosphatidylethanolamine, および triacylglycerol を分離した。それぞれの脂質クラスの脂肪酸組成を調べた結果，主な脂肪酸はパルミチン酸とエイコサペンタエン酸であったが，monogalactosyl diacylglycerol, phosphatidylcholine, phosphatidylethanolamine では炭素数 20 の高度不飽和脂肪酸の割合が高かった。また，いままでに *Porphyra* からは報告されていなかった *trans*-ω13-hexadecenoic acid が phosphatidylglycerol に，γ-リノレン酸が主として phosphatidylcholine と phosphatidylethanolamine に分布していることが明らかになった。(*143 東京都大田区大森東 5-4-6 山本海苔研究所，**153 目黒区駒場 3-8-1 東京大学教養学部，***444 岡崎市明大寺町西郷中 38 基礎生物学研究所，1)現勤務先 351-01 和光市広沢 2-1 理化学研究所太陽エネルギーグループ)