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Polyamine analysis of brown-algal seaweeds (class Phaeophyceae) from food markets —Distribution of diaminohexane, penta-amines, and hexa-amine—

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We bought samples of 16 species of brown macroalgal seaweeds (class Phaeophyceae) in the genera *Fucus*, *Ascophyllum*, *Sargassum*, *Saccharina*, *Undaria*, *Ecklonia*, *Eisenia*, *Chorda*, *Cladosiphon*, *Nemacystus*, *Petalonia*, and *Analipus* from food markets. We acid-extracted polyamines from them for analysis by high-performance liquid chromatography and high-performance gas chromatography-mass spectrometry, and estimated molar concentrations of the polyamines. In 12 of the species we found not only diaminopropane, putrescine, cadaverine, spermidine, norspermidine, homospermidine, spermine, norspermine, thermospermine, and agmatine, but also a novel long diamine, 1,6-diaminohexane. All species held caldopentamine and homocaldopentamine. Some species had minor contents of thermopentamine and caldohexamine. The brown-algal seaweeds are much richer in long linear polyamines than multicellular green- and red-algal seaweeds.

Key words: brown alga, diaminohexane, hexa-amine, penta-amine, Phaeophyceae, polyamine

INTRODUCTION

To reveal the phylogenetic significance of the distribution of polyamines to the secondary and tertiary endosymbiosis of unicellular green algae (phylum Chlorophyta) or red algae (phylum Rhodophyta) into other unicellular organisms, and to investigate the evolution of green and red algae into multicellular macroalgae, we have analyzed the polyamines of various unicellular microalgae (Hamana, 2008; Hamana & Matsuzaki, 1982, 1985; Hamana & Niitsu, 2006; Hamana *et al*., 1990, 2004a, 2004b, 2013, 2016b). Recently we reported the distribution of penta-amines and hexa-amines, in addition to diamines, triamines, and tetra-amines, in photosynthetic unicellular microalgae of the classes Bacillariophyceae and Raphidophyceae of the phylum Heterokontophyta (=Ochrophyta), which evolved after the secondary symbiosis of red alga within stramenopiles (Hamana *et al*., 2016b). We also found three penta-amines in the non-photosynthetic, heterotrophic unicellular labyrinthulomycetes (phylum Labyrinthulomycota) (Hamana *et al*., 2016b). Marine photosynthetic, multicellular brown macroalgae in the class Phaeophyceae are unique among heterokontophytes in developing into multicellular forms with differentiated tissues, and are important human foods. To determine the distribution of long polyamines within the Heterokontophyta, we used large-scale polyamine extraction and improved methods of high-performance liquid chromatography (HPLC) and high-performance gas chromatography (HPGC), to analyze polyamines acid-extracted from 27 brown-algal seaweeds (16 species) bought from food markets.

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MATERIALS AND METHODS

We bought Bladder wrack (powder of *Fucus vesiculosus* "Hibamata" harvested in the USA) and Algit (powder of *Ascophyllum nodosum* "Algit" harvested in Norway) from Nature's Way Products, Inc., Utah, USA and Shinkyo Industries Inc., Yamaguchi, Japan. We bought frozen, dried or salted "Akamoku" (*Sargassum horneri*), "Hiziki" (*Sargassum fusiforme*), "Ma-konbu" (*Saccharina japonica*), "Kagome-konbu"

(*Saccharina sculpera*), "Wakame" (*Undaria pinnatifida*), "Hirome" (*Undaria undarioides*), "Kajime" (*Ecklonia cava*), "Kurome" (*Ecklonia kurome*), "Arame" (*Eisenia bicyclis*), "Tsurumo" (*Chorda filum*), "Mozuku" (*Nemacystus decipiens*), "Okinawa-mozuku" (*Cladosiphon okamuranus*), "Matsumo" (*Analipus japonicus*), and "Habanori" (*Petalonia binghamiae*) from seafood markets in Japan (Table 1).

Rehydrated and washed whole algae (100–200 g wet weight) were homogenized in 5% to 10% perchloric acid. Extracts were applied to a cation-exchange resin column (Dowex 50WX8; 3 cm i.d. ×1 cm) and polyamines were eluted with 6M HCl. The concentrated polyamines were analyzed by HPLC through a short cation-exchange resin column (Hitachi 2619F=Hitachi 2720; 4 mm i.d. ×50 mm) using post-labeled fluorometry after heating with *o*-phthalaldehyde in Hitachi L6000 (Hamana *et al*., 2016a, 2016b). On the other hand, after heptafluorobutyrization (HFB) of the concentrated polyamines, polyamines were analyzed by HPGC on a SHIMADZU GC-17A and HPGC-mass spectrometry (HPGC-MS) on a JEOL JMS-700, equipped with a long Inert Cap 1MS capillary column (0.32 mm i.d. ×30 m, df 0.25 μm; GL Sciences) at a temperature program of 90 to 280℃ at 16℃/min (Furuchi *et al*., 2015a, 2015b; Niitsu *et al*., 2014). Mass spectra of the HFB-polyamine derivatives were drawn from previous studies (Furuchi *et al*., 2015a, 2015b; Hamana *et al*., 2016a, 2016b; Niitsu *et al*., 2014). Molar concentrations of cellular polyamines per gram wet weight of the starting algae were estimated from the HPLC and HPGC analyses using authentic polyamine standards, and are shown in Table 1.

RESULTS AND DISCUSSION

Recently, caldopentamine (3333) and homocaldopentamine (3334) have been found in some unicellular algae in the heterokontophyte classes Bacillariophyceae and Raphidophyceae (Hamana *et al*., 2016b). *N*-methylated polyamines were found in the Bacillariophyceae (Hamana *et al*., 2016b). Previously, we analyzed polyamines of some brown macroalgae such as *Acinetospora crinita*, *Sargassum thungergii*, and *Sargassum fulvellum* of the Phaeophyceae and several unicellular microalgae of the classes Chrysophyceae, Eustigmatophyceae, Pelagophyceae, Pinguiophyceae, Schizocladiophyceae and Xanthophyceae of the Heterokontophyta were by the old type of HPLC and standard type of GC used in our laboratory, but never detected long polyamines or *N*-methylated polyamines (Hamana & Matsuzaki, 1982; Hamana, 2008). Other studies of polyamines in macroalgae have been limited to analyses of putrescine (4), spermidine (34), and spermine (343) (Fuell *et al*., 2010; Schweikert & Burritt, 2015).

Here, using improved HPLC and HPGC methods, we obtained polyamine profiles of 16 typical brown-algal seaweed species in four orders of the Phaeophyceae (Fig. 1 and Table 1). All species contained the penta-amines caldopentamine (3333) and homocaldopentamine (3334), in addition to the triamines norspermidine (33), spermidine (34), and homospermidine (44), and the tetra-amines norspermine (333), spermine (343), and thermospermine (334). Thermopentamine (3343) was detected sporadically in the brown algae. These results suggest that penta-amines are widely distributed within the Heterokontophyta, and that an aminopropyl-transfere preferentially occurred into the terminal aminopropyl moiety of norspermine (333) and thermospermine (334) produced caldopentamine (3333) and homocaldopentamine (3334) in the multicellular class Phaeophyceae as well as the unicellular classes Bacillariophyceae and Raphidophyceae. Although homocaldohexamine (33334) and thermohexamine (33343) have been found in a diatom, *Synedra acus*, in the Bacillariophyceae (Hamana *et al*., 2016b), we detected caldohexamine (33333) as a minor polyamine component in the brown macroalgae *Sargassum horneri* and *Chorda filum* here. The levels of spermine (up to 1.20 μmol/g wet weight), thermospermine (up to 0.60 μmol/g wet weight), caldopentamine (up to 1.17 μmol/g wet weight), and homocaldopentamine (up to 1.17 μmol/g wet weight) in the multicellular photosynthetic brown algae, were higher than the levels (up to 0.07, up to 0.03, up to 0.15, and up to 0.04 μmol/g weight, respectively) in the unicellular photosynthetic diatoms (Hamana *et al*., 2016b), indicating that the brown-algal seaweeds are rich in long polyamines. On the other hand, non-photosynthetic, heterotrophic unicellular labyrinthulomycetes also contains caldopentamine (3333), homocaldopentamine (3334) and thermopentamine (3343) (Hamana *et al*., 2016b). These results suggeste the widespread distribution of penta-amines and hexa-amines within stramenopiles.

We detected 1,6-diaminohexane (6), a long linear diamine, in many brown algae. 1,3-Diaminopropane (3), putrescine (1,4-diaminobutane) (4), cadaverine

Table 1 Concentration of polyamines of brown algae from food markets

Taxon Phylum Heterokontophyta (Ochrophyta) Class Phaeophyceae	Commercial name in Japan	Product source	Sample No. and Preservation	Polyamines (μmol/g wet weight)														
				Dap 3	Put 4	Cad 5	Dah 6	NSpd 33	Spd 34	HSpd 44	NSpm 333	Spm 343	TSpm 334	CPen 3333	HCPen 3334	TPen 3343	CHex 33333	Agm
Oder Fucales																		
Fucus vesiculosus	"Hibamata"	USA	No. 1 Dry	0.20	0.95	0.10	0.02	0.13	0.50	0.29	0.73	0.28	0.60	0.15	0.28	-	-	-
		USA	No. 2 Dry	-	0.02	0.08	0.02	0.12	0.38	0.02	0.18	0.40	0.10	0.20	1.15	-	-	-
Ascophyllum nodosum	"Algit"	Norway	No. 1 Dry	0.25	0.40	0.85	-	0.06	0.25	-	0.14	0.02	0.03	-	0.07	-	-	-
		Norway	No. 2 Dry	0.10	0.30	1.05	-	0.08	0.30	0.01	0.05	0.02	0.04	0.01	0.46	-	-	-
Sargassum horneri	"Akamoku"	Shimane	No. 1 Dry	0.03	0.04	0.02	0.02	0.22	0.36	0.02	0.26	0.07	0.05	1.15	0.77	0.07	0.01	-
		Kanagawa	No. 2 Dry	0.25	0.10	0.15	0.08	0.25	0.90	0.01	0.15	0.20	0.03	1.17	0.10	-	-	-
Sargassum fusiforme	("Me-Hiziki")	Nagasaki	No. 1 Dry	-	0.05	0.42	-	0.20	0.23	0.05	0.03	0.15	0.02	0.01	0.01	-	-	0.05
(formerly *Hizikia fusiforme*)	("Naga-Hiziki")	Nagasaki	No. 2 Dry	0.01	0.17	0.40	0.03	0.03	0.25	0.01	0.01	0.48	0.02	0.01	0.05	-	-	-
	"Hiziki"	Chiba	No. 3 Dry	0.02	0.80	0.27	0.10	0.17	0.67	0.10	0.12	0.09	0.09	0.03	0.06	-	-	-
Order Laminariales																		
Saccharina japonica	"Ma-konbu"	Iwate	No. 1 Dry	0.02	0.06	-	0.04	0.10	0.50	0.44	0.06	1.20	0.01	0.98	1.07	0.01	-	-
(formerly *Laminaria japonica*)		Hokkaido	No. 2 Dry	-	0.02	-	0.01	0.09	0.09	-	0.03	0.18	0.01	1.20	0.45	-	-	-
Saccharina sculpera	"Kagome-konbu"	Hokkaido	Dry	-	0.02	-	-	0.03	0.07	-	0.07	0.20	0.01	1.15	0.13	-	-	-
Undaria pinnatifida	"Wakame"	Tokushima	No. 1 Salting	0.02	0.15	-	-	0.10	0.35	1.12	0.25	0.50	0.10	0.20	0.45	-	-	-
		Iwate	No. 2 Dry	0.20	0.05	-	-	0.30	0.50	0.15	0.78	0.85	0.20	0.82	0.85	-	-	-
	("Mekabu")	Mie	No. 3 Dry	-	0.01	-	-	0.02	0.52	-	0.10	0.59	0.03	1.05	1.16	-	-	-
Undaria undarioides	"Hirome"	Mie	Dry	0.02	0.04	0.10	0.05	0.15	1.20	0.13	0.12	0.50	0.02	0.40	0.20	-	-	-
Ecklonia cava	"Kajime"	Ishikawa	Dry	0.01	0.05	0.01	0.02	0.06	0.90	0.01	0.05	0.11	0.05	0.10	1.17	0.01	-	0.07
Ecklonia kurome	"Kurome"	Ohita	Dry	0.05	0.10	0.13	0.02	0.03	1.10	0.01	0.14	0.24	0.42	0.10	0.95	-	-	0.35
Eisenia bicyclis	"Arame"	Chiba	Dry	0.02	0.30	0.10	0.03	0.01	0.40	0.02	0.01	1.20	0.02	0.01	0.04	-	-	-
Chorda filum	"Tsurumo"	Ishikawa	Dry	0.01	0.01	-	0.04	0.05	0.09	-	0.13	0.66	0.02	0.15	0.70	0.01	0.01	-
Order Ectocarpales																		
Cladosiphon okamuranus	"Okinawa-mozuku"	Okinawa	No. 1 Dry	-	0.02	0.04	-	0.15	1.06	0.05	0.45	1.15	0.01	0.74	0.01	-	-	0.09
		Okinawa	No. 2 Freezing	0.01	0.15	0.10	0.15	0.12	0.90	0.13	0.18	1.00	0.01	0.66	0.01	0.01	-	0.06
		Okinawa	No. 3 Salting	-	-	-	0.01	0.14	0.27	0.17	0.18	0.83	0.01	1.15	0.01	-	-	-
Nemacystus decipiens	"Mozuku" ("Ito-mozuku")	Ishikawa	Salting	0.15	1.00	1.15	0.05	0.20	0.30	0.05	0.11	0.25	-	1.12	0.01	-	-	0.05
Petalonia binghamiae	"Habanori"	Chiba	Dry	0.04	0.06	0.02	0.02	0.06	0.18	0.01	0.10	0.09	0.01	1.15	0.02	0.05	-	-
Order Ralfsiales																		
Analipus japonicus	"Matsumo"	Iwate	No. 1 Dry	-	-	-	-	0.20	0.50	-	0.05	0.42	-	0.70	0.10	-	-	-
(formerly *Heterochordaria abietina*)		Iwate	No. 2 Dry	0.03	0.04	-	-	0.10	0.42	0.01	0.09	0.75	0.02	1.22	0.35	-	-	-

Dap (3) (=the numbers of methylene chain units (CH_2) between NH_2 or NH), diaminopropane; Put (4), putrescine; Cad (5), cadaverine; Dah (6), diaminohexane; NSpd (33), norspermidine; Spd (34), spermidine; HSpd (44), homospermidine; NSpm (333), norspermine; Spm (343), spermine; TSpm (334), thermospermine; CPen (3333), caldopentamine; HCPen (3334), homocaldopentamine, TPen (3343), thermopentamine; CHex (33333), caldohexamine; Agm, agmatine; -, not detected (<0.005 μmol/g wet weight). Methylated polyamines found in *Ascophyllum nodosum* and *Undaria pinnatifida* are excluded in Table 1.

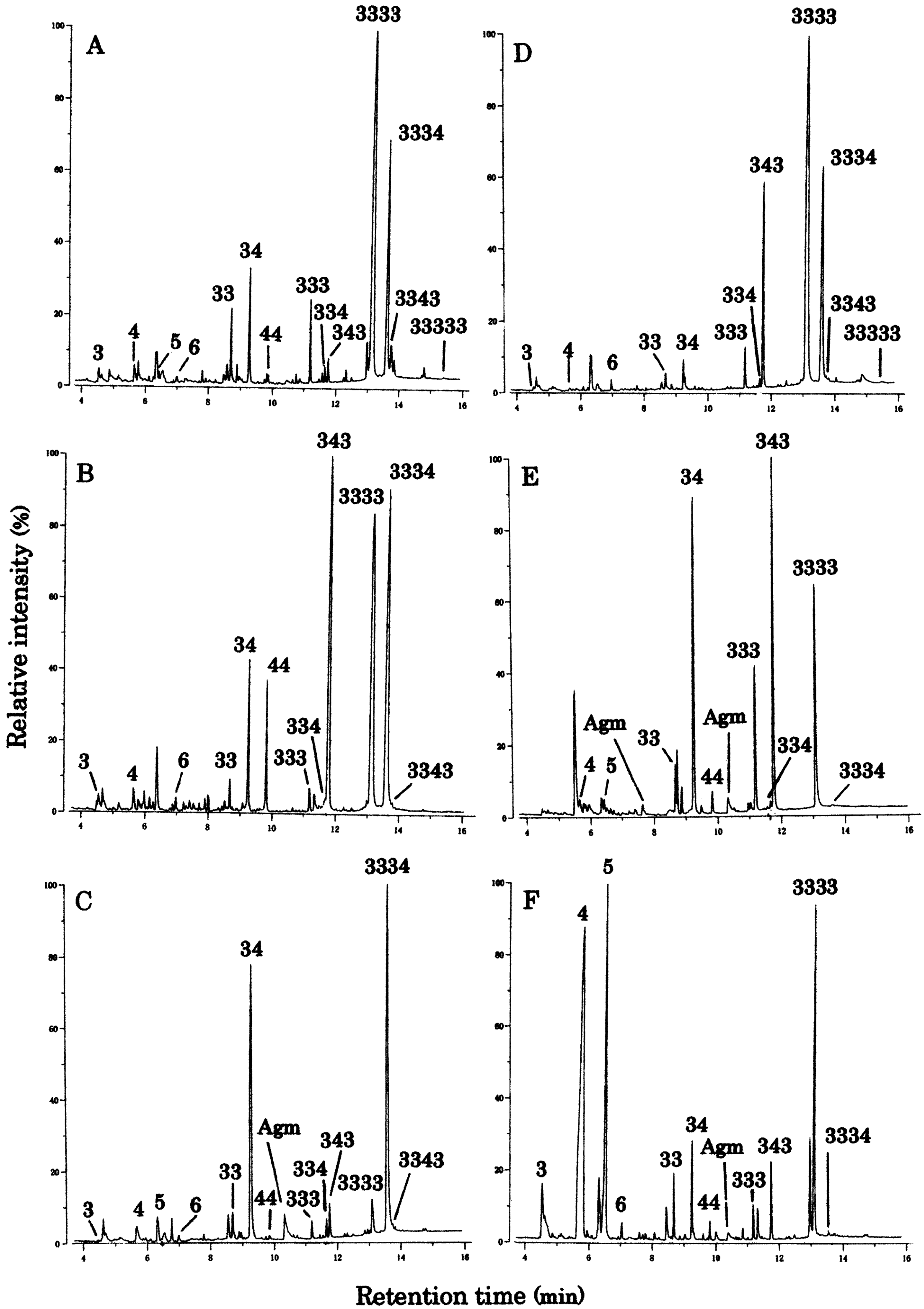

Fig. 1 HPGC spectra of polyamines of (A) *Sargassum horneri* No. 1, (B) *Saccharina japonica* No. 1, (C) *Ecklonia cava*, (D) *Chorda filum*, (E) *Cladosiphon okamuranus* No. 1, and (F) *Nemacystus decipiens*. Numeric codes are explained in Table 1. Agmatine (Agm) is destroyed during HPGC analysis. Other non-amine peaks were also detected.

(1,5-diaminopentane) (5), agmatine and homoagmatine are produced respectively from L-2,4-diaminobutylic acid, L-ornithine, L-lysine, L-arginine, and L-homoarginine by decarboxylases in many microorganisms and plants, and 1,3-diaminopropane can be produced from norspermidine, spermidine, norspermine, spermine, or thermospermine by polyamine oxidases in algae and plants (Fuell *et al.*, 2010; Schweikert & Burritt, 2015). Ours is the first report of the natural occurrence of 1,6-diaminohexane as a biological aliphatic diamine. Biological decarboxylation of L- (or D-) homolysine (2,7-diaminoheptanoic acid), a non-natural amino acid, to produce 1,6-diaminohexane is unknown.

The *N*-methylated diamines methyldiaminopropane (M3), methylcadaverine (M5), and dimethylputrescine (M4M) were detected in *Undaria pinnatifida* (0.03 μmol/g wet weight of M3) and *Ascophyllum nodosum* (0.12 μmol/g wet weight of M5, 0.10 μmol/g wet weight of M4M). Mono-methylated or di-methylated triamines, tetra-amines and penta-amines found in some unicellular diatoms of the Bacillariophyceae (Hamana *et al.*, 2016b) were not detected in the 16 brown-algal species of the Phaeophyceae. In diatoms, long-chain polyamines (up to 20 repeated units of *N*-methyl-propylamine or propylamine) were associated with silica during biomineralization (Sumper & Lehmann, 2006; Sumper & Brunner, 2008).

The occurrence of 1,6-diaminohexane and polycationic linear penta-amine and hexa-amine in the marine brown algae might be related to their abundance of polyanionic acidic-polysaccharides such as alginic acid, fucoidan, and ascophyllan. Since significant amounts of penta-amine and hexa-amine have never been found in multicellular green- and red-algal seaweeds, their discovery in brown algae will contribute nutritional use of brown-algal seaweeds.

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食品市場由来の海産褐藻類のポリアミン分析
—ジアミノヘキサン，ペンタアミンおよびヘキサアミンの分布—

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大型海産褐藻類の 16 種（ヒバマタ，アルギット，アカモク，ヒジキ，マコンブ，カゴメコンブ，ワカメ，ヒロメ，カジメ，クロメ，アラメ，ツルモ，モズク，オキナワモズク，マツモ，ハバノリ）を市場より入手し，過塩素酸抽出後のポリアミン濃縮画分を高性能液体クロマトグラフィー（HPLC）と高性能ガスクロマトグラフィー・質量分析（HPGC-MS）にて分析し，藻体湿重量あたりのモル濃度を算出した．12 種において，ジアミノプロパン，プトレッシン，カダベリン，スペルミジン，ノルスペルミジン，ホモスペルミジン，スペルミン，ノルスペルミン，サーモスペルミン，アグマチンに加えて，生物試料では初めての長鎖ジアミンである 1,6–ジアミノヘキサンを検出した．全 16 種がカルドペンタミンとホモカルドペンタミンを含有していた．数種がサーモペンタミンやカルドヘキサミンをも有していた．褐藻類は，多細胞性（大型）の海産紅藻類や海産緑藻類に比べて，長直鎖型ポリアミンに富んでいる．