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Inorganic ion compositions in brown algae, with special reference to sulfuric acid ion accumulations

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Abstract

Cellular pH estimated from cell extract pH and the ion compositions of major inorganic ions (Na^+, $NH_4{}^+$, K^+, Mg^{2+}, Ca^{2+},Cl^-, Br^-, $NO_3{}^-$, $SO_4{}^{2-}$) were studied by ion chromatography in 61 species of 10 orders (Dictyotales, Desmarestiales, Ectocarpales, Chordariales, Scytosiphonales, Dictyosiphonales, Cutleriales, Sporochnales, Laminariales and Fucales) of Phaeophyceae. Three species in the order Dictyotales, *Dictyopteris* sp., *Spatoglossum solierii* (Chauv.) Kützing and *Zonaria stipitata* Tanaka et K. Nozawa, were newly found to be highly acidic (pH 0.6 and 1.4 within cells), in addition to previously reported dictyotalean species, *Dictyopteris latiuscula* (Okamura) Okamura, *D. prolifera* (Okamura) Okamura, *D. repens* (Okamura) Børgesen and *Spatoglossum crassum* J. Tanaka. They all contained high concentrations of $SO_4{}^{2-}$ perhaps within the vacuoles. Furthermore, *Delamarea attanuata* (Kjellman) Rosenvinge (Dictyosiphonales) and *Thalassiophyllum clathrus* (Gmel.) P. *et* R. (Laminariales) were shown to contain relatively high concentrations of $SO_4{}^{2-}$ balanced by relatively high concentrations of Ca^{2+}.

Introduction

Species of the order Desmarestiales (Phaeophyceae) are well known to accumulate highly acidic substances in vegetative cells of the sporophytes. The pH of the cell extracts have been shown to be as low as 2.0 (Wirth & Rigg, 1937; Eppley & Bovell, 1958; Schiff, 1962; McClintock et al., 1982; Sasaki et al., 1999), and the cellular pH has been estimated to be ca. 0.5–0.9 (McClintock et al., 1982; Sasaki et al., 1999). The acidic substance was thought to be sulfuric acid stored in vacuoles of the sporophytic cells (Eppley & Bovell, 1958; McClintock et al., 1982). Recently, Sasaki et al. (1999) reported a similar phenomenon in some species of Dictyotales; four species of *Dictyopteris* and *Spatoglossum* were found to be highly acidic due to high concentrations of cellular $SO_4{}^{2-}$. The authors showed, by ion chromatography of major inorganic ions, that the sum of metal cations was significantly lower than that of anions, and the extremely low pH indicated that the excess of anions is balanced by protons (H^+). On the other hand, Sasaki et al. (1999) also found that some non-acidic dictyotalean species (*Dictyota dichotoma* (Hudson) Lamouroux, *Padina minor* Yamada, etc.) contained relatively high concentrations of $SO_4{}^{2-}$, which was balanced by high concentrations of Mg^{2+} and so did not cause high acidity. The authors therefore suggested that the inorganic ion composition could be rather variable depending on the species.

In this work, we aimed to extend the survey of inorganic ion compositions to cover additional brown algae in other orders, and to explore the diversity of inorganic ion compositions in brown algae.

Materials and methods

Selected members of the following orders were examined for cell extract pH and inorganic ion compositions by ion chromatography: Dictyotales, Desmarestiales, Ectocarpales, Chordariales, Scytosiphonales,

Table 1. Taxonomic list of species examined in the present survey and their origins

Species	Collection sites and dates
Dictyotales	
Dictyopteris divaricata (Okamura) Okamura	Sasaki et al. (1999)
D. latiuscula (Okamura) Okamura	Sasaki et al. (1999), Yamaguchi Pref. (13 June 1997)
D. prolifera (Okamura) Okamura	Sasaki et al. (1999), Awaji Isl., Hyogo Pref. (10 Jan., 7 July, 28 Oct., 12 Dec., 1997, 27 Feb., 28 Apr., 17 July 1998)
D. repens (Okamura) Børgesen	Sasaki et al. (1999)
D. undulata Holmes	Sasaki et al. (1999), Awaji Isl., Hyogo Pref. (17 Dec. 1998); Kinosaki, Hyogo Pref. (11 Oct. 1998)
Dictyopteris sp.	Ishigaki Isl., Okinawa Pref. (21 Apr. 1999)
Dictyota dichotoma (Hudson) Lamouroux	Sasaki et al. (1999), Awaji Isl., Hyogo Pref. (3 July, 12 Dec. 1997, 28 Apr., 17 July, 3 Dec. 1998)
D. divaricata Lamouroux	Sasaki et al. (1999)
D. linearis (C. Agardh) Greville	Sasaki et al. (1999)
Dictyotopsis propagulifera Troll	Sasaki et al. (1999)
Dilophus okamurae Dawson	Sasaki et al. (1999), Awaji Isl., Hyogo Pref. (17 Dec. 1998); Seto, Wakayama Pref. (10 May 1997)
Distromium decumbens (Okamura) Levring	Sasaki et al. (1999), Kasumi, Hyogo Pref. (11 Oct. 1998)
Lobophora variegata (Lamouroux) Womersley	Sasaki et al. (1999)
Pachydictyon coriaceum (Holmes) Okamura	Sasaki et al. (1999)
Padina arborescens Holmes	Sasaki et al. (1999), Awaji Isl., Hyogo Pref. (3 Dec. 1998)
P. crassa Yamada	Sasaki et al. (1999)
P. minor Yamada	Sasaki et al. (1999)
Padina sp.	Sasaki et al. (1999)
Spatoglossum crassum J. Tanaka	Sasaki et al. (1999), Awaji Isl., Hyogo Pref. (10 Jan. 1997)
S. pacificum Yendo	Sasaki et al. (1999), Awaji Isl., Hyogo Pref. (27 Aug. 1997)
S. solierii (Chauv.) Kützing	Tarifa Island, Cadiz, Spain (28 Aug. 1999)
Stypopodium zonale (Lamouroux) Papenfuss	Sasaki et al. (1999)
Taonia leunebackerae Farlow	Sasaki et al. (1999)
Zonaria diesingiana J. Agardh	Sasaki et al. (1999)
Z. stipitata Tanaka et K. Nozawa	Kerama Islands, Okinawa Pref. (8 Mar. 1999)
Desmarestiales	
Desmarestia aculeata (L.) Lamouroux	Sasaki et al. (1999)
D. latifrons Kützing	Kawai culture
D. ligulata (Stackhouse) Lamouroux	Sasaki et al. (1999)
D. tabacoides Okamura	Sasaki et al. (1999), Maiko, Hyogo Pref. (3 Mar. 1999)
D. viridis (Müller) Lamouroux	Sasaki et al. (1999)
Ectocarpales	
Bodanella lauterbornii Zimmermann	D.G. Müller culture
Chordariales	
Papenfussiella kuromo (Yendo) Inagaki	Awaji Isl., Hyogo Pref. (17 Dec. 1998)
Saundersella simplex (Saunders) Kylin	Abacha Bay, Kamchatka, Russia (24 July 1998)
Ishige okamurae Yendo	Awaji Isl., Hyogo Pref. (17 Dec. 1998)
I. sinicola (Setchell et Gardner) Chihara	Awaji Isl., Hyogo Pref. (17 Dec. 1998)
Leathesia difformis (Linnaeus) Areschoug	Awaji Isl., Hyogo Pref. (1 July 1999)
Scytosiphonales	
Colpomenia sinuosa (Mertens *ex* Roth) Derbes et Solier	Awaji Isl., Hyogo Pref. (3 Dec. 1998)
Petalonia binghamiae (J. Agardh) Vinogradova	Awaji Isl., Hyogo Pref. (17 Dec. 1998)
Scytosiphon gracilis Kogame.	Awaji Isl., Hyogo Pref. (17 Dec. 1998)

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Table 1. Continued

Species	Collection sites and dates
Dictyosiphonales	
Delamarea attenuata (Kjellman) Rosenvinge	Hokkaido Pref. (June 1997)
Stschapovia flagellaris A. D. Zinova	Kawai culture
Dictyosiphon foeniculaceus (Hudson) Greville	Abacha bay, Kamchatka, Russia (28 July 1998)
Cutleriales	
Cutleria cylindrica Okamura	Mijo, Korea (3 March 1998)
C. multifida (Turner) Greville	Ibota, Yamaguchi Pref. (12 June 1997)
Microzonia velutina (Harv.) J. Ag.	D.G. Müller culture
Sporochnales	
Carpomitra costata (Stackhouse) Batters	Cheju Isl., Korea (10 Feb. 1998)
Sporochnus scoparius Harvey	Ibota, Yamaguchi Pref. (12 June 1997)
Laminariales	
Agarum clathratum Dumortier	Muroran, Hokkaido Pref. (28 Feb. 1999)
Chorda filum (Linnaeus) Stackhouse	Ohzuchi, Miyagi Pref. (11 June 1998)
Ecklonia cava Kjellman	Awaji Isl., Hyogo Pref. (3 Dec., 17 Dec. 1998)
Ecklonia kurome Okamura	Takeno, Hyogo Pref. (2 July 1999)
Kjellmaniella crassifolia Miyabe	Muroran, Hokkaido Pref. (28 Feb. 1999)
Thalassiophyllum clathrus (Gmel.) P. et R.	Abacha Bay, Kamchatka, Russia (24 July 1998)
Fucales	
Hizikia fusiformis (Harvey) Okamura	Awaji Isl., Hyogo Pref. (3 Dec. 1998)
Sargassum confusum C. Agardh	Takeno, Hyogo Pref. (2 July 1999)
S. hemiphyllum (Turner) C. Agardh	Awaji Isl., Hyogo Pref. (3 Dec. 1998); Takeno, Hyogo Pref. (11 Oct. 1997)
S. micracanthum (Kützing) Endlicher	Awaji Isl., Hyogo Pref. (1 July 1999)
S. patens C. Agardh	Takeno, Hyogo Pref. (2 July 1999)
S. ringgoldianum ssp. *coreanum* (J. Agardh) Yoshida	Awaji Isl., Hyogo Pref. (17 Dec. 1998)
S. thunbergii (Mertens *ex* Roth) Kuntze	Awaji Isl., Hyogo Pref. (3 Dec. 1998)
Turbinaria ornata (Turner) J. Agardh	Ishigaki Isl., Okinawa Pref. (22 Jan. 1998)

Dictyosiphonales, Cutleriales, Sporochnales, Laminariales and Fucales. Origins of the materials are listed in Table 1, but only additional materials added after Sasaki et al. (1999) are listed for the following taxa: *Dictyopteris divaricata*, *D. latiuscula*, *D. prolifera*, *D. repens*, *D. undulata*, *Dictyota dichotoma*, *D. divaricata*, *D. linearis*, *Dictyotopsis propagulifera*, *Dilophus okamurae*, *Distromium decumbens*, *Lobophora variegata*, *Pachydictyon coriaceum*, *Padina arborescens*, *P. crassa*, *P. minor*, *Padina* sp., *Spatoglossum crassum*, *S. pacificum*, *S. solierii*, *Stypopodium zonale*, *Taonia leunebackerae*, *Zonaria diesingiana* (Dictyotales), *Desmarestia aculeata*, *D. latifrons*, *D. ligulata*, *D. tabacoides*, *D. viridis* (Desmarestiales). Field materials collected were transported as quickly as possible to the laboratory in plastic containers filled with seawater kept at 10–15 °C, and promptly used for the analyses. Unialgal culture strains were cultured in PESI medium (Tatewaki, 1966) under a 16:8 h LD cycle illuminated with daylight-type white fluorescent lighting of approximately 50 μmol $m^{-2}s^{-1}$ at 10 °C. Sea water samples for ion-chromatography were collected from several localities at Awaji Isl., Hyogo Pref. (3, 17 July, 16 December 1997) and Ibota, Yamaguchi Pref. (12, 13 June 1997), and their data of ion-chromatography ($N = 7$) were averaged.

The pH measurements and ion chromatography of the cell extracts were done following the protocol in Sasaki et al. (1999) except for *Bodanella lauterbornii*, *Ishige okamurae* and *I. sinicola*. These species were highly tolerant to freshwater treatment because *B. lauterbornii* is a freshwater species, and *Ishige* spp. are upper subtidal species. Therefore, for the extractions of these species, fresh algal tissue was soaked in 1 ml (when the sample weighed less than 60 mg) or 2 ml (when the sample weighed more than 60 mg) boiling distilled water (ca. 100 °C) in a 1.5 ml (or 2.0 ml) microfuge tube for 60 min using a block incubator. The sample tissue was then removed and the extract was passed through an ultra-filtration filter and used for both analyses.

Intracellular pH and concentrations of inorganic ions were estimated from values of the extracts based on the following calibration formula:

$$\text{Estimated intracellular pH} = -\log\,[\alpha \times \text{dilution factor}],$$

where α is proton concentration (M) of the extract calculated from the pH measurement data, and the dilution factor = [distilled water (g) + sample fresh weight (g) × water content (ml)] / sample fresh weight (g) × water content (ml).

Results

pH measurements

The pH values measured for the algal tissue extracts of various taxa are shown in Table 2. Among Dictyotales, in addition to *Dictyopteris latiuscula*, *D. prolifera*, *D. repens* and *Spatoglossum crassum*, an unidentified species of *Dictyopteris* collected from Ishigaki Island, *Zonaria stipitata* and *Spatoglossum solierii* were newly found to be highly acidic, with an estimated cellular pH of approximately 0.6–1. Among Desmarestiales, *Desmarestia ligulata*, *D. tabacoides* and *D. viridis* showed extremely low intracellular pH, but no other comparably acidic species were found in the eight other orders examined.

Ion chromatography

The ionic compositions of 60 phaeophycean species from 10 orders are shown in Table 2. Seawater contained approximately 360 mM Na^+ and 380 mM Cl^- as primary ions, and 40 mM Mg^{2+} and 26 mM $SO_4{}^{2-}$ as secondary major ions (Table 2, Fig. 1a).

Among Dictyotales and Desmarestiales, acidic species (*Dictyopteris latiuscula*, *D. prolifera*, *D. repens*, *Dictyopteris* sp., *Spatoglossum crassum*, *S. solierii*, *Desmarestia ligulata*, *D. tabacoides* and *D. viridis*) contained significantly high concentrations of $SO_4{}^{2-}$ (ca. 140–340 mM) (Fig. 1b). *Zonaria stipitata* also contained relatively high concentrations of $SO_4{}^{2-}$ (65.4 mM).

In contrast, most non-acidic species of Dictyotales and Desmarestiales contained only 2.1–49.1 mM $SO_4{}^{2-}$, which was similar to that of seawater (26.3 mM) (Fig. 1c). Exceptionally, *Dictyota dichotoma* (78.7 mM), *D. divaricata* (108.9 mM), *Distromium decumbens* (65.8 mM), *Padina arborescens* (67.7 mM), *P. crassa* (175.4 mM), *P. minor* (193.0 mM) contained relatively high concentrations of $SO_4{}^{2-}$ (Fig. 1d). These species contained relatively high concentration of Mg^{2+} (60.4–184.1 mM) as counter ion. Outside these orders, *Leathesia difformis* (Chordariales), *Sargassum confusum* and *S. patens* (Fucales) included relatively high concentration of Mg^{2+} (61.0–69.0 mM).

As seen in Table 2, the sum of cations and anions are not perfectly equal even in non-acidic species because not all of the inorganic ions and none of the organic ions were measured by ion chromatography. However, they are more or less equal in non-acidic species, compared with the acidic species listed above. A large part of the difference between total anions and cations in acidic species is considered to be protons, which could not be measured by ion chromatography. This high concentration of protons causes the extremely low pH in the acidic species.

Although most other non-acidic species in the other orders contained relatively low concentrations of $SO_4{}^{2-}$ (2.1–60.4 mM), *Delamarea attenuata* (Dictyosiphonales, 274.7 mM) and *Thalassiophyllum clathrus* (Laminariales, 284.0 mM) contained considerably higher concentrations of $SO_4{}^{2-}$ (Fig. 1e, f). As counter ions, *Leathesia difformis* and *Dictyosiphon foeniculaceus* contained relatively, high concentration of Mg^{2+} (respectively 66.5 and 97.3 mM). However, *Delamarea attenuata*, *Agarum clathratum* and *Thalassiophyllum clathrus* contained relatively high concentration of Ca^{2+} (76.5–258.9 mM). The only freshwater alga *Bodanella lauterbornii* also contained relatively high concentration of Ca^{2+} (92.1 mM).

Among other minor ions, *Padina* sp. and *Microzonia velutina* (Dictyotales) contained relatively high concentration of $NH_4{}^+$ (42.9–44.2 mM), whereas most other species contained less than 10 mM. *Kjellmania crassifolia* (Laminariales) contained high concentration of NO^{3-} (96.5 mM), whereas most other species contained less than 30 mM.

Table 2. Estimated cellular pH and inorganic ion composition in the species examined. Each datum of the inorganic ion represents average concentration ± S.E. (mM)

Species	Extract pH	pH within cells ± S.D.	No. of samples (*n*)	Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	Br^-	NO_3^-	SO_4^{2-}	Σ cation	Σ anion	Σ anion / Σ cation	No. of samples (*n*)
Dictyotales																
Dictyopteris divaricata	5.3–5.7	4.2±0.27	5	77.3	0.8	164.8	5.8	0.9	184.9	22.4	N.D.	28.9	256.4	265.2	1.03	2
D. latiuscula	1.7–3.4	0.7±0.23	54	195.1±22.8	6.7±3.7	178.5±18.5	36.2±3.9	32.4±4.0	201.6±28.7	1.8±1.7	N.D.	187.0±18.4	518.4±35.7	577.4±51.0	1.11	9
D. prolifera	1.7–4.3	0.8±0.52	160	156.6±10.4	5.7±0.6	181.3±12.2	30.4±1.4	25.9±2.0	160.5±14.6	0.8±0.5	0.2±0.1	214.9±7.1	456.3±19.5	591.3±25.2	1.30	62
D. repens	3.4	0.8	1	161.7	16.6	151.1	54.8	121.7	182.4	2.4	44.1	277.5	682.5	783.8	1.15	1
D. undulata	5.4–7.3	4.2±0.79	43	142.8±19.5	1.6±0.7	243.1±17.2	18.5±2.4	4.1±1.3	351.1±22.2	1.2±0.8	1.0±0.4	17.8±2.0	432.8±28.1	388.8±23.9	0.90	20
Dictyopteris. sp.	2.9–3.7	1.1±0.31	10	93.6±8.6	2.4±0.8	331.2±23.6	28.7±3.1	28.4±3.5	317.3±19.2	N.D.	10.4±2.5	138.3±18.6	541.3±40.9	614.7±49.5	1.14	10
Dictyota dichotoma	5.6–7.2	4.5±0.39	123	94.8±6.8	1.1±0.4	204.5±12.6	83.4±10.6	5.7±0.7	284.6±12.3	0.6±0.3	0.6±0.2	78.7±8.7	478.2±19.1	443.3±19.1	0.93	76
D. divaricata	6.2–6.5	4.7±0.09	5	57.4	1.2	82.9	86.1	11.1	95.6	0.8	N.D.	108.9	336.0	314.2	0.94	2
D. linearis	5.9–6.4	4.9±0.17	8	133.1	1.9	374.6	17.8	4.1	428.7	20.5	N.D.	34.3	553.1	517.7	0.94	2
Dictyotopsis propagulifera	6.9–7.3	5.1±0.00	4	62.3±2.8	2.4±1.6	64.6±6.3	3.0±1.0	2.1±0.9	40.0±5.8	0.1±0.1	35.7±9.8	1.3±0.8	139.5±11.4	78.4±11.0	0.56	4
Dilophus okamurae	5.9–7.3	5.2±0.67	33	148.5±36.1	0.5±0.3	197.4±16.2	13.0±5.0	4.1±3.0	296.5±61.8	1.5±0.7	N.D.	22.0±3.5	380.7±64.6	342.0±65.8	0.90	9
Distromium decumbens	6.6–8.1	5.1±0.84	10	92.7±11.2	3.5±2.8	104.2±23.0	66.8±18.6	14.2±4.0	132.0±16.8	N.D.	1.1±0.8	65.8±15.0	362.3±26.4	264.6±30.7	0.73	6
Lobophora variegata	6.8–8.9	5.5±0.68	15	134.5±12.6	N.D.	97.6±24.8	11.9±4.0	2.5±0.7	175.6±33.1	0.7±0.7	N.D.	23.4±3.4	261.0±33.9	223.2±39.3	0.86	3
Pachydictyon coriaceum	5.1–6.3	4.4±0.46	19	232.0±87.5	0.3±0.3	381.0±95.8	44.7±14.3	8.5±4.2	618.9±161.1	5.6±3.7	0.5±0.5	33.5±11.2	719.8±188.6	692.0±185.9	0.96	4
Padina arborescens	6.6–7.9	5.7±0.53	31	175.3±30.4	N.D.	206.6±23.8	60.4±16.4	9.3±2.3	376.4±49.2	2.5±1.4	1.9±0.8	67.7±13.4	521.1±70.2	516.2±73.3	0.99	15
P. crassa	7.1–8.2	5.8±0.31	27	66.6±5.8	0.9±0.5	137.3±16.0	184.1±44.8	25.0±4.8	175.2±23.2	0.3±0.2	22.8±10.9	175.4±46.6	623.0±106.0	549.2±96.5	0.88	8
P. minor	7.9–8.4	6.5±0.19	22	75.2±12.0	1.5±1.0	98.9±24.1	159.4±37.0	24.2±2.6	110.7±10.6	0.9±0.95.7±1.8	193.0±24.7	542.7±62.1	503.4±57.1	0.93	6	
Padina sp.	6.4–6.7	4.5±0.56	5	72.3±9.4	44.2±20.0	166.9±25.6	44.0±9.8	4.4±1.1	235.1±21.4	0.2±0.1	16.3±3.0	44.1±4.7	380.4±38.2	339.9±33.0	0.89	5
Spatoglossum crassum	1.4–3.4	0.5±0.31	27	124.5±11.6	3.0±0.5	204.4±24.2	29.1±2.7	28.8±3.4	148.2±13.5	2.2±2.1	1.9±0.8	256.4±18.0	426.2±25.4	664.9±43.6	1.56	17
S. pacificum	5.0–6.6	4.4±0.03	43	92.5±8.6	0.1±0.1	298.9±30.1	9.8±2.31.9±0.4	324.1±38.1	1.0±0.5	5.1±2.4	23.5±2.7	414.9±37.1	377.2±36.6	0.91	10	
S. solierii	2.2–2.4	0.6±0.17	4	101.3±11.9	3.9±1.2	171.1±24.6	22.8±1.6	14.9±0.4	187.4±7.7	3.9±2.0	0.5±0.3	253.7±36.9	351.7±18.4	699.2±79.0	1.99	4
Stypopodium zonale	4.4–6.2	3.3±0.62	19	49.9±10.7	1.6±0.7	112.0±24.4	184.6±32.7	12.4±2.5	374.7±44.7	0.4±0.4	4.7±1.4	32.1±6.3	557.6±103.1	444.0±54.9	0.80	6
Taonia leunebackerae	6.1–6.2	4.4±0.21	2	72.5	1.2	116.0	31.8	4.4	68.3	1.0	9.8	49.1	262.1	177.4	0.68	2
Zonaria diesingiana	6.7–8.4	5.3±0.58	23	80.0±4.2	0.6±0.4	138.0±35.4	3.2±0.8	0.9±0.5	135.1±26.8	0.6±0.3	5.0±1.9	15.5±1.9	226.9±34.9	171.8±26.6	0.76	10
Z. stipitata	2.9–3.9	1.4±0.28	5	78.8±14.5	5.3±1.3	267.7±80.0	15.3±1.8	12.4±1.9	318.8±86.4	N.D.	N.D.	65.4±3.1	407.2±89.1	449.6±83.2	1.10	5
Desmarestiales																
Desmarestia aculeata	6.5–6.8	5.1±0.12	8	73.2±1.0	1.3±1.3	147.0±6.2	0.5±0.5	N.D.	212.2±14.2	0.2±0.2	N.D.	2.1±0.7	222.4±7.6	216.7±12.8	0.97	4
D. latifrons	7.1	4.6	1	143.8	8.0	34.7	36.3	3.0	104.1	N.D.	N.D.	11.1	265.0	126.2	0.48	1
D. ligulata	2.0–2.7	0.8±0.17	6	299.7±84.8	2.6±2.6	240.4±58.6	68.3±18.5	68.3±24.1	313.2±83.5	4.1±3.0	N.D.	349.7±112.6	821.8±222.5	1016.7±292.9	1.24	3
D. tabacoides	1.9–2.6	0.7±0.18	18	118.9±16.9	2.8±1.1	98.3±9.9	23.0±2.9	13.1±2.8	110.1±14.2	N.D.	1.3±0.5	175.4±12.9	292.4±35.5	462.2±35.5	1.58	10
D. viridis	1.3–2.4	0.5±0.12	16	86.2±17.9	1.0±0.8	136.5±18.5	23.6±3.7	30.7±6.0	114.8±25.7	14.0±12.4	15.9±15.5	249.1±30.1	332.3±53.9	642.9±88.0	1.93	4
Ectocarpales																
Bodanella lauterbornii	7.0	4.8	1	146.1	15.6	157.8	23.7	92.1	155.4	16.8	45.3	45.3	551.1	308.0	0.56	1

Continued on p. 258

Table 2 Continued

Species	Extract pH	pH within cells ± S.D.	No. of samples (*n*)	Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	Br^-	NO_3^-	SO_4^{2-}	Σ cation	Σ anion	Σ anion / Σ cation	No. of samples (*n*)
Chordariales																
Papenfussiella kuromo	6.3–6.4	4.5±0.22	4	127.3±7.5	N.D.	193.1±13.3	10.6±0.4	0.3±0.3	267.4±10.6	N.D.	0.6±0.6	8.5±0.9	342.3±19.3	285.1±11.0	0.83	4
Saundersella simplex	6.3	5.1±0.21	2	199.5	N.D.	66.5	19.7	3.6	276.4	1.5	N.D.	11.6	312.6	301.6	0.96	2
Ishige okamurae	5.4–5.8	4.0±0.18	5	293.5±6.6	3.3±1.1	108.5±4.3	34.2±4.4	16.1±3.2	244.2±15.0	1.1±0.9	0.9±0.9	15.6±1.4	505.9±14.2	277.4±18.8	0.55	5
I. sinicola	5.8–6.3	4.4±0.24	5	223.2±25.7	3.9±2.0	76.5±10.5	30.1±4.4	15.3±2.9	179.9±20.5	0.8±0.6	9.2±1.0	10.1±25.2	394.5±39.2	210.1±23.1	0.53	5
Leathesia difformis	6.5–6.9	5.4±0.25	4	222.4±62.5	N.D.	280.1±64.7	66.5±29.5	1.8±1.0	531.5±59.0	4.3±2.6	4.7±2.9	60.4±1.4	639.1±89.8	661.1±103.5	1.03	4
Scytosiphonales																
Colpomenia sinuosa	5.5–6.2	4.0±0.29	4	128.8±21.1	0.8±0.8	433.0±42.4	10.0±2.2	1.5±0.9	550.5±59.5	0.4±0.4	1.9±1.4	11.0±1.8	585.7±57.6	574.8±61.3	0.98	4
Petalonia binghamiae	6.1–6.4	4.7±0.10	4	60.9±5.3	N.D.	200.8±18.4	5.3±0.7	0.2±0.2	183.5±13.8	N.D.	12.8±1.2	37.0±6.5	272.7±21.4	270.3±25.4	0.99	4
Scytosiphon gracilis	6.2–6.3	4.8±0.08	4	98.9±18.7	N.D.	169.7±14.6	6.2±1.1	0.2±0.2	189.9±17.4	N.D.	10.7±2.1	38.3±2.8	281.3±21.4	277.1±21.5	0.99	4
Dictyosiphonales																
Delamarea attenuata	6.3–6.9	4.8±0.21	6	166.0	3.1	244.7	39.5	125.1	217.7	19.4	5.5	274.7	743.0	792.2	1.07	2
Stschapovia flagellaris	6.6–7.1	4.8±0.99	3	151.3±66.0	N.D.	240.1±58.5	8.9±1.9	2.2±2.2	251.8±39.7	N.D.	N.D.	33.0±6.0	413.6±13.0	318.0±51.2	0.77	3
Dictyosiphon foeniculaceus	6.3–6.4	4.4±0.20	3	150.4±14.0	3.9±0.9	183.9±19.4	97.3±13.4	8.6±3.2	387.7±41.9	4.3±2.2	N.D.	55.1±7.0	550.0±64.8	502.1±56.4	0.91	3
Cutleriales																
Cutleria cylindrica	5.7–5.9	3.8±0.144	126.0	7.2	338.6	2.7	3.3	263.8	N.D.	N.D.	20.1	483.8	304.1	0.63	2	
C. multifida	6.5–7.1	5.4±0.42	3	294.9	N.D.	121.0	25.2	5.2	440.7	21.2	N.D.	7.0	827.0	475.9	0.58	2
Microzonia velutina	6.6	5.0±0.12	3	25.9±8.1	42.9±3.4	63.0±3.7	8.7±0.3	1.3±0.6	47.9±8.9	3.9±0.5	31.3±3.9	1.3±0.8	152.0±11.2	85.7±13.4	0.56	3
Sporochnales																
Carpomitra costata	6.8–7.0	4.7±0.15	6	115.0±24.8	8.7±2.2	592.6±95.6	12.9±8.5	17.7±17.7	516.5±157.1	6.9±6.9	N.D.	11.1±1.9	777.6±172.7	546.0±160.0	0.70	3
Sporochnus scoparius	6.5–6.6	5.3±0.20	3	68.5	N.D.	256.3	6.3	N.D.	319.7	9.2	1.9	4.1	337.0	339.0	1.01	1
Laminariales																
Agarum clathratum	6.1–6.3	4.7±0.14	6	154.1±23.4	5.2±2.0	211.7±17.2	26.2±2.1	76.5±14.9	467.4±37.6	N.D.	18.6±4.5	52.4±13.6	576.2±31.9	590.9±38.0	1.03	4
Chorda filum	4.8–5.6	3.8±0.14	14	258.4±38.7	N.D.	354.4±41.7	21.9±3.4	4.9±1.0	461.1±60.4	0.2±0.2	N.D.	15.6±2.2	666.4±85.1	492.5±61.7	0.74	4
Ecklonia cava	5.8–6.4	4.8±0.25	5	168.6±14.7	2.0±2.0	132.5±16.3	5.0±0.6	1.0±0.4	276.0±36.6	0.3±0.2	4.9±2.7	8.3±0.7	315.3±32.7	297.8±40.6	0.94	5
Ecklonia kurome	6.1–6.3	4.6±0.24	4	157.7±10.2	N.D.	282.3±28.2	10.7±1.9	2.8±1.0	386.7±38.4	1.4±0.5	0.8±0.4	7.0±1.2	466.9±40.3	402.9±40.6	0.86	4
Kjellmaniella crassifolia	6.1–6.3	4.9±0.05	6	206.0±17.9	1.2±1.2	226.2±26.4	7.4±1.9	3.0±0.4	320.1±42.3	N.D.	96.5±29.7	9.2±0.8	454.3±10.7	435.1±21.7	0.96	4
Thalassiophyllum clathrus	6.3	4.9±0.06	3	132.9±38.7	N.D.	158.4±52.5	47.4±19.0	258.9±97.9	242.8±67.3	N.D.	N.D.	284.0±88.6	903.9±323.6	810.0±243.3	0.90	3
Fucales																
Hizikia fusiformis	6.3–6.9	5.1±0.17	4	112.3±5.0	N.D.	388.4±22.1	6.0±1.2	0.9±0.6	476.3±23.4	0.2±0.2	1.2±1.2	4.0±1.5	515.0±27.9	485.8±24.0	0.94	4
Sargassum confusum	5.0–5.6	3.7±0.30	4	99.6±5.4	N.D.	296.1±8.0	69.0±3.2	11.3±1.1	498.5±13.0	1.3±1.3	13.2±2.1	3.1±0.3	556.3±10.4	519.3±11.1	0.93	4
S. hemiphyllum	5.8–6.5	4.4±0.29	10	111.9±17.3	0.7±0.7	227.5±52.4	11.0±2.7	0.3±0.3	238.4±46.9	N.D.	N.D.7.1±1.4	363.0±44.1	252.6±47.2	0.70	4	
S. micracanthum	5.7–6.1	4.0±0.17	4	165.0±4.7	2.2±2.2	320.0±35.0	39.8±11.0	2.5±2.5	473.4±57.0	0.3±0.3	20.8±8.2	4.5±1.8	571.9±61.8	503.5±67.2	0.88	4
S. patens	5.5–5.8	3.9±0.18	4	93.1±12.1	N.D.	360.1±39.8	61.0±4.4	2.5±1.0	489.2±48.0	12.5±2.0	34.5±2.3	6.3±1.6	580.4±55.7	548.6±45.2	0.95	4
S. ringgoldianum ssp. *coreanum*	5.8–6.04.2±0.31	4	146.4±2	1.2	N.D.	222.1±29.9	18.8±3.8	2.1±0.8	379.8±55.3	N.D.	N.D.	3.6±1.8	410.0±56.3	387.0±55.5	0.94	4
S. thunbergii	6.2–6.9	5.0±0.34	4	98.5±6.3	N.D.	179.7±10.6	4.7±0.5	0.4±0.2	256.9±16.9	0.1±0.1	1.8±0.9	4.2±1.0	288.0±16.8	267.3±18.1	0.93	4
Turbinaria ornata	6.3–7.0	5.1±0.36	13	85.0	N.D.	108.4	1.7	N.D.	138.9	N.D.	N.D.	0.6	197.0	140.0	0.71	2
Sea Water				360.1±41.3	N.D.	7.5±0.8	38.9±5.1	7.8±1.2	379.6±60.2	0.9±0.2	N.D.	26.3±1.5	460.9±54.7	433.1±61.1	0.97	7
Sea Water Medium				441.5±0.9	N.D.	11.2±0.6	50.4±0.6	5.9±1.8	494.7±11.0	N.D.	N.D.	25.9±1.0	565.3±5.1	547.0±12.9	0.94	4
Fresh water medium				2.40±0.02	N.D.	0.02±0.00	0.04±0.00	0.5±0.00	1.02±0.02	N.D.	1.00±0.00	0.12±0.00	3.5±0.02	2.25±0.02	0.64	4

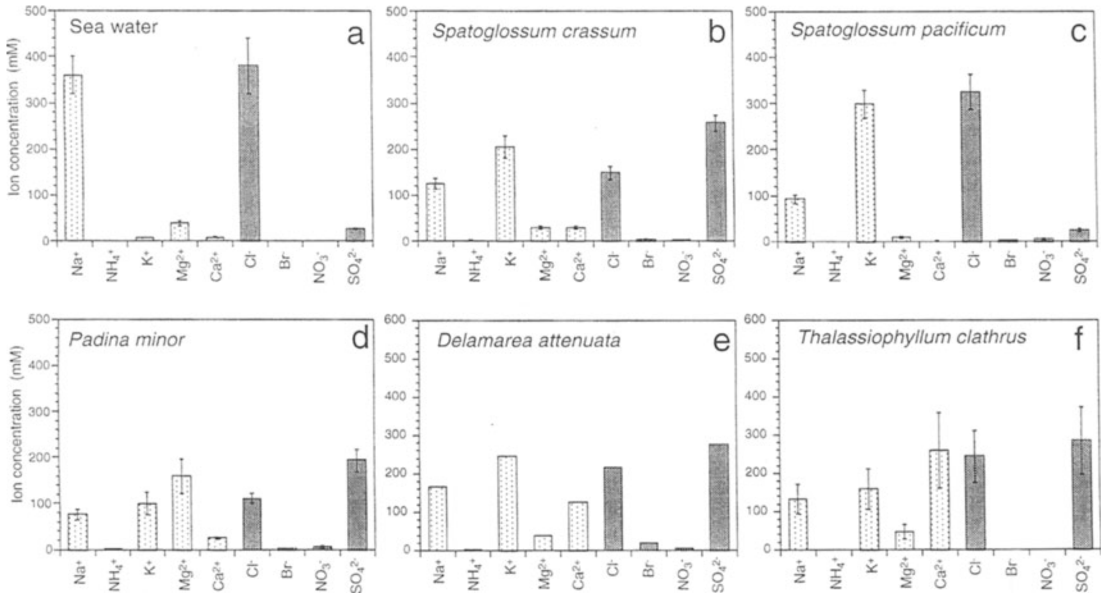

Figure 1. Estimated intracellular ion concentrations (mM) of representative acidic and non-acidic species: *Spatoglossum crassum* (b, acidic with high $SO_4{}^{2-}$), *Spatoglossum pacificum* (c, non-acidic), *Padina minor* (d, non-acidic with high $SO_4{}^{2-}$ and Mg^{2+}), *Delamarea attenuata* (e, non-acidic with high $SO_4{}^{2-}$ and Ca^{2+}) and *Thalassiophyllum clathrus* (f, non-acidic with high $SO_4{}^{2-}$ and Ca^{2+}). Ionic compositions of sea water (average of 7 samples) are also shown (a). For number of samples in each species and the data for other species, see Table 2.

Discussion

In the present survey, three dictyotalean species (*Dictyopteris* sp., *Spatoglossum solierii* and *Zonaria stipitata*) were newly found to be highly acidic, accumulating high concentrations of $SO_4{}^{2-}$ although the concentration was not very high in *Z. stipitata*. Their cellular pH was comparable to previously reported acidic species of Dictyotales and Desmarestiales (McClintock et al., 1982; Sasaki et al., 1999). Therefore, highly acidic species in the order Dictyotales are distributed among three genera (*Dictyopteris*, *Spatoglossum* and *Zonaria*), although not all members of these genera showed high acidity. This situation clearly contrasts with Desmarestiales, in which the monophyly of this character is supported by molecular phylogenetic studies (Peters et al., 1997). The order Dictyotales has been classified into two families based on the number of apical cells (Dictyotaceae with a single apical cell and Zonariaceae with marginal rows of apical cells, J. Agardh 1894), or into two tribes (Dictyoteae Greville and Zonarieae De Toni) in a single family Dictyotaceae (Womersley, 1987). Among these, all genera containing highly acidic species belong to the family/tribe Zonariaceae/Zonarieae. Accumulation of sulfate ion itself is found in much broader systematic groups within Dictyotales (Sasaki et al., 1999) in both of the families/tribes, and other orders (e.g., Dictyosiphonales and Laminariales; present paper). However, high acidity caused by balancing the high concentration of sulfate ion by protons (H^+) might have occurred after the evolutionary divergence of the family/tribe within Dictyotales, as occurred relatively newly in Desmarestiales (Peters et al., 1997). This question would be clarified by molecular phylogenetic analysis of the related genera in Dictyotales that is in progress in our laboratory.

Delamarea attenuata, growing on exposed upper subtidal and lower intertidal rocks in North Pacific and North Atlantic cold waters, has been noted to be relatively delicate and easily damaged when collected and kept in small containers filled with culture medium (H. Kawai, unpublished observations). In such cases, the medium turns yellow from the leakage of some substances from the plants (presumably carotenoids). Such vulnerability of the plants might be explained by the accumulation of a high concentration of sulfate ion. When *Delamarea* plants suffer some environmental changes, sulfate ion might leak to cause further damage to the cytoplasm and the whole cell suffers (sometimes lethal) damage, causing the leakage of yellow substances.

Well-developed vacuoles are one of the most important cellular organelles characterizing plant cells. Their functions have been relatively well studied in higher plant cells, but very little is known for algal cells. Vacuoles of various macro-algal cells have similar structures as those of higher plants and also contribute to production of turgor pressure. However, there could be considerable differences in other functional aspects, because the environmental conditions surrounding the cells are very different. The new finding of accumulation of relatively high concentrations of Ca^{2+} in addition to Mg^{2+}, both functioning as counter ions of $SO_4{}^{2-}$, implies that the active accumulation of sulfuric acid itself is not the sole function. It may be a result of complex regulation of various correlated cations and anions in brown algae. As for higher plants, the relationship between $SO_4{}^{2-}$ and Ca^{2+} has been reported in cultured tobacco cells (Smith, 1978; Jones & Smith, 1981). The transport of $SO_4{}^{2-}$ was stimulated by Ca^{2+} and increased the ratio of $SO_4{}^{2-}$ in cell. However, the quantity of intracellular $SO_4{}^{2-}$ reported was 0.01–10.0 mM, and the quantity of Ca^{2+} used in the experiment was 0.5 mM (Jones & Smith, 1981), much lower than that of accumulated ions in brown algae.

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