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ACCUMULATION OF SULFURIC ACID IN DICTYOTALES (PHAEOPHYCEAE): TAXONOMIC DISTRIBUTION AND ION CHROMATOGRAPHY OF CELL EXTRACTS[1]

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Four species in the order Dictyotales (*Dictyopteris latiuscula* (Okamura) Okamura, *D. prolifera* (Okamura) Okamura, *D. repens* (Okamura) Børgesen, and *Spatoglossum crassum* J. Tanaka) were found to be highly acidic as in some species of the order Desmarestiales (Phaeophyceae). The pH within their cells, presumably that of the vacuole, was estimated to be 0.5 to 0.9 by pH measurements of their cell extracts in distilled water. However, other species of these genera (*D. divaricata* (Okamura) Okamura, *D. undulata* Holmes, and *S. pacificum* Yendo) did not show high acidity. Ion chromatography of the cell extracts showed that those species contained high concentrations of $SO_4{}^{2-}$ within their cells, up to 10 times that in seawater but relatively low Cl^-. The sum of cations examined (Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+}) was significantly lower than that of anions (Cl^-, Br^-, NO_3^-, $SO_4{}^{2-}$), and the difference is presumed to represent protons (H^+), causing the extremely low cell sap pH. Estimated cellular proton concentrations calculated from the pH data roughly agreed with those calculated from differences between the sum of cations and anions and that of anions. Although certain other, nonacidic, dictyotalean species also contained high concentrations of $SO_4{}^{2-}$, these species contained high concentrations of Mg^{2+}, and the sums of cations and anions were balanced.

Key index words: ***Dictyopteris;*** **Dictyotales; ion chromatography; low pH; *Spatoglossum;* sulfuric acid**

Many species of the order Desmarestiales (Phaeophyceae) are known to accumulate highly acidic substances within the vegetative cells of the sporophytes. This phenomenon has attracted many physiologists, and a number of papers have been published on this topic (Wirth and Rigg 1937, Kylin 1938, Blinks 1951, Miwa 1953, Meeuse 1956, Eppley and Bovell 1958, Shiff 1962, McClintock et al. 1982). The cells discharge the vacuolar acidic substance or substances following damage by drying because of extreme low tides or stranding or when they are soaked in freshwater. The pH of the cell extracts from such damaged sporophytes becomes as low as 2.0. By calculating a dilution rate on the basis of the volume of cells and the dilution medium, McClintock et al. (1982) estimated the pH within the cells as 0.74 in *Desmarestia ligulata* (Stackhouse) Lamouroux and 0.89 in *D. viridis* (Müller) Lamouroux. The acidic pH is considered to be due to sulfuric acid stored in vacuoles of the sporophytic cells (Eppley and Bovell 1958). On the basis of their molecular phylogenetic study, Peters et al. (1997) suggested that cellular accumulation of such extremely high levels of sulfuric acid evolved within the order Desmarestiales only once and that they did so relatively recently.

We noticed a similar phenomenon in some species of Dictyotales (e.g. *Dictyopteris* spp. and *Spatoglossum crassum* Tanaka) in which damaged plants quickly turned green. Although this peculiar phenomenon of color change following damage was noticed earlier, its occurrence within the species of the order and the mechanism that underlies it have not been studied. We presumed that it is also due to high concentrations of free sulfuric acid stored within the vacuoles, as in cells of desmarestialean species. However, if this is the case, this peculiar character must have evolved at least twice in the Phaeophyceae, although it is not known in any other seaweed lineages (e.g. Chlorophyta and Rhodophyta). Therefore, in this study we examined the pH and ion compositions of extracts from various dictyotalean species to compare them with those of the Desmarestiales and to explore their phylogenetic implications.

MATERIALS AND METHODS

Materials. The algae used in the study and information on their acquisition are shown in Table 1. Algae were transported to the laboratory in plastic containers filled with 100–3000 mL of seawater and maintained at 10–15° C for pH measurements and extractions for ion chromatography. Occasionally, the pH measurements and the extractions were done immediately after the collection at the collection site to avoid any possible damage that might be caused by transportation. Unialgal culture strains were

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TABLE 1. Collection data of the samples used for pH study and ion-chromatography. Collection sites are in Japan unless otherwise noted.

Species	Collection sites and dates
Dictyotales	
Dictyopteris divaricata (Okamura) Okamura	Ibota, Yamaguchi Prefecture (12 June 1997)
Dictyopteris latiuscula (Okamura) Okamura	Ibota, Yamaguchi Prefecture (12 June 1997); Awaji Island, Hyogo Prefecture (10 Apr., 24 May[a], 25 May, 13 June, 2 Sep. 1997[a])
Dictyopteris prolifera (Okamura) Okamura	Kasumi, Hyogo Prefecture (20 Apr.[a], 13 Sep. 1997[a]), Awaji Island, Hyogo Prefecture (10 Apr., 13 May[a], 25 May[a], 3 July[a], 17 July, 27 Aug., 2 Sep., 16 Dec. 1997[b]); Seto, Wakayama Prefecture (10 May 1997[a]); Cheju Island (11 Feb. 1998[a]), Pusan, Korea (14 Oct. 1997)
Dictyopteris repens (Okamura) Børgesen	Ishigaki Island, Okinawa Prefecture (22 Jan. 1998)
Dictyopteris undulata Holmes	Amamioshima Island, Okinawa Prefecture (16 June 1997[a]), Ibota, Yamaguchi Prefecture (12 June 1997), Takeno, Hyogo Prefecture (13 Sep 1997), Awaji Island, Hyogo Prefecture (3 July 1997[a], 28 Apr. 1998); Seto, Wakayama Prefecture (10 May 1997, 29 May 1998)
Dictyopteris propagulifera Troll	Kamiya culture (Fiji)
Dictyota dichotoma (Hudson) Lamouroux	Amamiohshima Island, Okinawa Prefecture (16 June 1997[a]), Takeno, Hyogo Prefecture (20 Apr. 1997); Awaji Island, Hyogo Prefecture (10 Apr., 13 May, 17 July, 2 Sep., 28 Oct. 1997, 24 Feb. 1998[b]); Cheju Island, Korea (9 Feb. 1998[a])
Dictyota divaricata Lamouroux	Yura, Hyogo Prefecture (2 Sep. 1997)
Dictyota linearis (C. Agardh) Greville	Ibota, Yamaguchi Prefecture (12 June 1997), Awaji Island, Hyogo Prefecture (27 Aug. 1997[a])
Dilophus okamurae Dawson	Awaji Island, Hyogo Prefecture (10 Apr., 13 May, 13 June, 3 July, 17 July 1997, 27 Feb., 17 July 1998), Seto, Wakayama Prefecture (10 May 1997[a])
Distromium decumbens (Okamura) Levring	Cheju Island, Korea (10 Feb. 1998)
Lobophora variegata (Lamouroux) Womersley	Ishigaki Island, Okinawa Prefecture (21 Jan., 22 Jan. 1998[a])
Pachydictyon coriaceum (Holmes) Okamura	Ibota, Yamaguchi Prefecture (12 June 1997), Awaji Island, Hyogo Prefecture (17 July 1997, 27 Feb. 1998[a]), Seto, Wakayama Prefecture (10 May 1997)
Padina arborescens Holmes	Ibota, Yamaguchi Prefecture (12 June 1997), Awaji Island (13 May[a], 3 July, 27 Aug. 1997[a], 27 Feb. 1998), Seto, Wakayama Prefecture (10 May 1997)
Padina crassa Yamada	Ishigaki Island, Okinawa Prefecture (21 Jan. 1998), Takeno, Hyogo Prefecture (13 Sep. 1997); Cheju Island, Korea (10 Feb. 1998)
Padina minor Yamada	Ishigaki Island, Okinawa Prefecture (22 Jan. 1998); Seto, Wakayama Prefecture (29 May 1998)
Padina sp.	D. G. Müller culture
Spatoglossum crassum J. Tanaka	Awaji Island, Hyogo Prefecture (10 Apr., 16 Dec. 1997[a], 27 Feb. 1998); Pusan, Korea (4 Oct. 1997)
S. pacificum Yendo	Awaji Island, Hyogo Prefecture (10 Apr., 13 May, 17 July 1997, 28 Apr. 1998), Pusan, Korea (4 Oct. 1997)
Stypopodium zonale (Lamouroux) Papenfuss	Ishigaki Island, Okinawa Prefecture (22 Jan. 1998); Seto, Wakayama Prefecture (29 May 1998)
Taonia lennebackerae Farlow	D.G. Müller culture
Zonaria diesingiana J. Agardh	Takeno, Hyogo Prefecture (13 Sep. 1997), Seto, Wakayama Prefecture (29 May 1998) Cheju Island, Korea (10 Feb. 1998)
Desmarestiales	
Desmarestia aculeata (L.) Lamouroux	Abacha Bay, Kamchatka, Russia (28 July 1998)
Desmarestia ligulata (Stackhouse) Lamouroux	Muroran, Hokkaido (10 July 1997)
Desmarestia tabacoides Okamura	Maiko, Hyogo Prefecture (2 May 1998)
Desmarestia viridis (Müller) Lamouroux	Ibota, Yamaguchi Prefecture (12 June 1997), Awaji Island, Hyogo Prefecture (10 Apr., 24 May 1997)

[a] Samples used only for pH study.

[b] Samples used only for ion chromatography.

propagated in plastic dishes containing 50 mL of PESI medium (Tatewaki 1966) under culture conditions of 16:8 h LD (light: dark) cycle illuminated with daylight-type white fluorescent lighting of about 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 10° C. Seawater samples for ion chromatography were collected from several localities at Awaji Island, Hyogo prefecture(3 and 17 July and 16 December 1997) and Ibota, Yamaguchi prefecture (12 and 13 June 1997), Japan, and ion chromatography data ($n = 7$) for these were averaged.

pH measurements of the cell extracts. Extraction procedures for pH measurements basically followed McClintock et al. (1982). Seawater was carefully removed from the surface of the sample tissue with filter paper (Paper Filter No. 1, Advantec Toyo, Tokyo). Fresh algal tissue of 0.01–0.10 g was soaked without agitation in 0.5 mL distilled water (pH 6.1–6.5) in a 1.5-mL microfuge tube at room temperature for 15 min. The extract was pipetted to a new microfuge tube and its pH measured using a portable pH meter (B-212 Twin-pH, Horiba, Tokyo). After the extraction experiments, the samples were weighed after drying at 70° C for 12 h. Cellular pH was estimated from pH values of the extract on the basis of the following calibration formula of dilution: Dilution factor = (distilled water [g] + sample fresh weight [g] × water content)/sample fresh weight (g) × water content.

Ion chromatography of the cell extracts. Seawater on the surface of the thallus was carefully wiped off with pieces of filter paper (Pa-

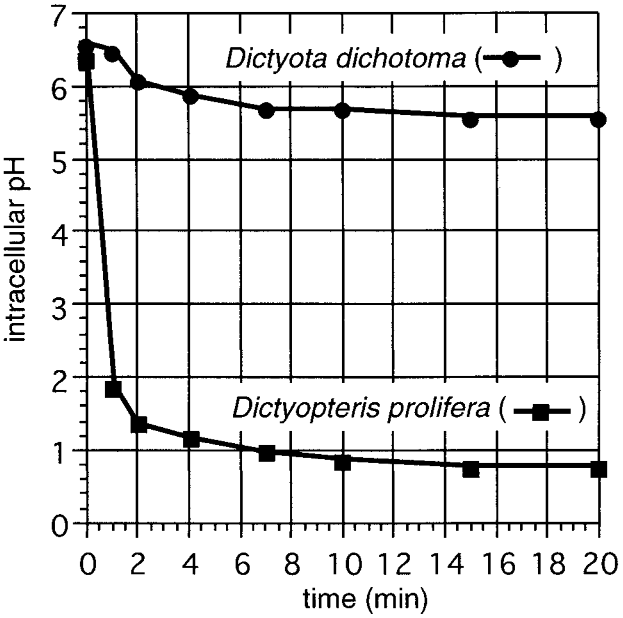

FIG. 1. Time course of acid leakage from *Dictyopteris prolifera* (acidic species) and *Dictyota dichotoma* (nonacidic species) in distilled water. Each pH datum is calibrated by dilution formula (see Materials and Methods).

per Filter No. 1). To remove remaining seawater on the surface, the tissue was quickly rinsed with 1 M sorbitol in a Buchner funnel with gentle suction. Immediately after the rinse, the fresh tissue was weighed, and each sample was soaked in 1 mL (when the sample weighed less than 60 mg) or 2 mL (when the sample weighed more than 60 mg) of distilled water in a 1.5- or 2-mL microfuge tube and extracted at room temperature for 40 min. The tissue was then removed, and the extract was passed through an ultrafiltration filter (Molcut filter, UFP1 LCC24, Nihon Millipore Inc., Tokyo) and used for analysis.

Using a (flow) path-switchable ion chromatography system equipped with conductivity detectors (ICA-5000, Toa Electronics, Tokyo) and HPLC-packed columns (PCI-201S for anion analysis and PCI-311 for cation analysis), inorganic ions in the tissue extract were measured, and intracellular concentrations were reconstructed by multiplying by the dilution factors. Analyses of NH_4^+, K^+, Mg^{2+}, and Ca^{2+} were conducted with a solvent mixture of 2.5 mM phthalate + 2.4 mM Tris-hydoroxy-methyl-amino-methane; for Cl^-, Br^-, NO_3^-, and SO_4^{2-}, 12 mM tartaric acid was used as the elution solvent.

RESULTS

pH Measurements

Preliminary experiments that varied the extraction period in distilled water with selected acidic and nonacidic species (*Dictyopteris prolifera* and *Dictyota dichotoma*) showed that dictyotalean species generally suffered lethal damage when soaked in distilled water for 10–15 min and that the leakage of acids from cells reached saturation within 15 min (Fig. 1). Therefore, we chose an extraction time of 15 min in subsequent experiments. The pH values measured for the algal tissue extracts of various taxa are shown in Table 2. Among Dictyotales, extremely low intracellular pH (less than 1 within cells) was demonstrated in *Dictyopteris latiuscula, Dictyopteris prolifera, Dictyopteris repens,* and *Spatoglossum crassum.* The intracellular pH of other members of these genera, such as *Dictyopteris divaricata, Dictyopteris undulata,* and *Spatoglossum pacificum,* was between 4.2 and 4.4. Intracellular pH was not low in species of the genera *Dictyota, Dilophus, Lobophora, Pachydictyon, Padina, Stypopodium, Taonia,* and *Zonaria* that were studied in the present survey (pH 4.3–6.5, excluding *Stypopodium zonale*). Some specimens of *Stypopodium zonale* had relatively low intracellular pH (3.3) compared with other nonacidic species. Acids were not discharged from these species even after prolonged periods of extraction (≤ 2 h). Acidic species of *Desmarestia* (*D. ligulata, D. tabacoides,* and *D. viridis*) showed an intracellular pH as low as 0.5–0.8, whereas that of *D. aculeata* (nonacidic species) was only 5.1.

TABLE 2. The pH of the extract and estimated intracellular pH.

Taxa	Extract pH	pH within cells	(±SD)	Number of samples
Dictyotales				
Dictyopteris divaricata	5.3–5.7	4.2	(±0.27)	5
Dictyopteris latiuscula	1.7–3.4	0.9	(±0.23)	34
Dictyopteris prolifera	1.7–3.3	0.8	(±0.52)	77
Dictyopteris repens	3.4	0.8	—	1
Dictyopteris undulata	5.4–7.3	4.4	(±0.63)	38
Dictyota dichotoma	5.9–7.2	4.8	(±0.35)	40
Dictyota divaricata	6.2–6.5	4.7	(±0.09)	5
Dictyota linearis	5.9–6.4	4.9	(±0.17)	8
Dictyotopsis propagulifera	7.1–7.3	5.1	—	2
Dilophus okamurae	5.9–7.3	5.2	(±0.67)	33
Distromium decumbens	4.6–5.5	5.0	(±0.34)	7
Lobophora variegata	6.8–8.9	5.5	(±0.68)	15
Pachydictyon coriaceum	5.1–6.3	4.4	(±0.46)	19
Padina arborescens	6.6–7.9	5.8	(±0.52)	27
Padina crassa	7.1–8.2	5.8	(±0.31)	27
Padina minor	7.9–8.4	6.5	(±0.19)	22
Padina sp.	6.6–6.7	4.3	—	2
Spatoglossum crassum	1.4–3.4	0.5	(±0.31)	27
Spatoglossum pacificum	5.0–6.6	4.4	(±0.03)	43
Stypopodium zonale	4.4–6.2	3.3	(±0.62)	19
Taonia leunebackerae	6.1–6.2	4.4	—	2
Zonaria diesingiata	6.7–8.4	5.3	(±0.58)	23
Desmarestiales				
Desmarestia acleata	6.5–6.8	5.1	(±0.12)	8
Desmarestia ligulata	2.0–2.7	0.8	(±0.17)	6
Desmarestia tabacoides	1.9–2.6	0.8	(±0.15)	12
Desmarestia viridis	1.3–2.4	0.5	(±0.12)	16

Ion chromatography

Figure 2 demonstrates that intracellular inorganic ions of a selected acidic dictyotalean species (*Dictyopteris prolifera*) were extracted fully 30–40 min after transfer to distilled water. This indicated that 40 min of extraction was sufficient in all species to prepare specimens for ion chromatography (see Materials and Methods). The ionic compositions of 22 dictyotalean species from 12 genera and four desmarestialean species (one genus) are summarized in Table 3. The total positive charges, Σ cations ($=\Sigma\ c_i z_i$); negative charges, Σ anions ($=-\Sigma\ c_i z_i$); and their ratios, Σ anions /Σ cations are listed at the

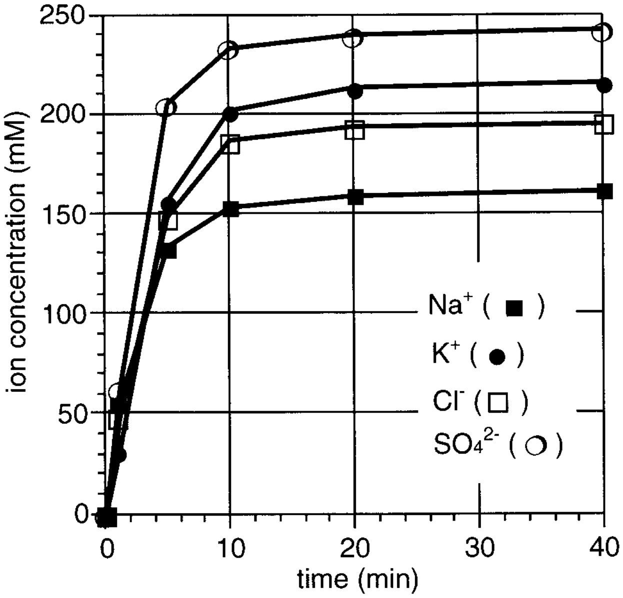

FIG. 2. Time course of extraction in distilled water of major ions (Na^+, K^+, Cl^-, and SO_4^{2-}) from *Dictyopteris prolifera* (acidic species). Data based on average of two measurements.

right side of Table 3, where c_i is the concentration of each given ion and z_i is the valence of the ion. Because the total positive charges must balance the total negative charges, any difference between the two values indicated that some ion(s) was (were) not accounted for by ion chromatographic analysis. An anion deficit of several 10's to 100 equivalents has frequently been observed in many algae and higher plants, and this gap was thought to be due either to negatively charged, low molecular weight, organic compounds or to a fixed negative charge of the tonoplast membrane (Kirst and Bisson 1979, Kirst 1990). However, the large cation deficit found in the present study suggested a contribution to the electrical neutrality by H^+, which was not measurable with ion chromatography. Therefore, the Σ anions /Σ cations value higher than unity indicated acidic species. Intracellular proton concentrations in acidic species estimated from ion chromatography data (Σ anions$-\Sigma$ cations) generally agreed with those estimated from intracellular pH data (Table 4).

In Figure 3, the ionic composition of some representative species and seawater are shown graphically. The ionic compositions of eight seawater samples, collected from three locations in the Seto Inland Sea contained about 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. All acidic species of Dictyotales (*Dictyopteris latiuscula, D. prolifera, D. repens,* and *Spatoglossum crassum*) and Desmarestiales (*Desmarestia ligulata, D. tabacoides,* and *D. viridis*) contained significantly elevated concentrations of SO_4^{2-} (ca. 180–340 mM). By contrast, nonacidic species (*Dictyopteris divaricata, Dictyopteris undulata, Dictyota linearis, Dilophus okamurae, Distromium decumbens, Lobophora variegata, Pachydictyon cariaceum, Spatoglossum pacificum, Stypopodium zonale,* and *Desmarestia aculeata*) contained only 2.1–35.2 mM SO_4^{2-}, which was similar to that of seawater (26.3 mM). Exceptionally, *Dictyota dichotoma* (105.5 mM), *Dictyota divaricata* (108.9 mM), *Padina crassa* (175.4 mM), and *P. minor* (193.0 mM) contained relatively high concentrations of SO_4^{2-}, although the cellular pH value was not highly acidic (4.7–8.5). Table 5 shows the contribution of each cation and anion to the respective charges. Note that all acidic algal species contain more Mg^{2+} and SO_4^{2-} compared to K^+, Na^+, and Cl^-.

DISCUSSION

Four dictyotalean species were found in this study to be highly acidic, as are some desmarestialean species, and their cellular pH was comparable to that of *Desmarestia* spp. (McClintock et al. 1982). This suggests that this trait must have evolved multiple times in the Phaeophyceae because the two orders are considered to be phylogenetically distant by both conventional and molecular systematics (Bold and Wynne 1985, van den Hoek et al. 1995, Tan and Druehl 1993). However, in contrast to the Desmarestiales, in which monophyly of acidic species has been suggested (Peters et al. 1997), dictyotalean acidic species belong to two separate genera (*Dictyopteris* and *Spatoglossum*), whereas other species of both genera were not acidic. Does this mean that the evolution of high acidity evolved multiple times within the Dictyotales? Two explanations are possible: 1) It evolved separately in the two genera *Dictyopteris* and *Spatoglossum* in Dictyotales, or 2) it evolved only once in the Dictyotales, as in the Desmarestiales, and the generic separation of these species must be reconsidered. However, the ion chromatography also suggests a third possibility (see the following discussion), namely, that acid accumulation is a more complex character.

The comparison of the sum of all cations and anions examined in the present study revealed the following features. 1) In seawater the sum of cations is slightly higher than that of anions (Table 3; Σ anions/Σ cations: 0.94). 2) In highly acidic species the sum of anions is considerably higher than that of cations (Σ anions/Σ cations is higher than 1, except for *Desmarestia tabacoides*). The difference is presumed to be due to protons (H^+), which cannot be determined with ion chromatography, and this per se is the cause of very low cellular pH (Table 4). The reason that the Σ anions/Σ cations ratio was relatively low in *D. tabocoides* is unclear, but the sample could have suffered some damage during transportation (no on-site extractions could be done for the species). 3) In all other nonacidic species the sum of cations is comparable to or a little higher than that of anions (Σ anions/Σ cations: ca. 0.8–1.0). The Σ anions/Σ cations ratio is considerably lower in some species compared to seawater or to

Table 3. Intracellular ionic concentrations of various dictyotalean and desmarestialean species and the ionic composition of seawater. Data calculated from the ionic concentrations of cell extracts and the dilution formula. Sum of total positive and negative charges and their ratios are listed on right side columns. Each datum represents average concentration ± SE (mM).

Species	Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}
Dictyotales					
Dictyopteris divaricata	77.3	0.8	164.8	5.8	0.9
D. latiuscula[a]	181.0 (±28.2)	2.1 (±1.5)	186.7 (±38.2)	35.2 (±6.9)	37.1 (±6.0)
D. prolifera[a]	157.4 (±14.7)	6.4 (±0.9)	147.1 (±11.6)	29.3 (±1.9)	20.3 (±2.4)
D. repens[a]	161.7	16.6	151.1	54.8	121.7
D. undulata	155.8 (±38.5)	2.2 (±1.3)	227.5 (±17.7)	18.0 (±4.9)	5.1 (±2.7)
Dictyota dichotoma	90.6 (±10.5)	1.4 (±0.7)	173.3 (±14.5)	103.0 (±15.8)	5.9 (±1.2)
D. divaricata	57.4	1.2	82.9	86.1	11.1
D. linearis	133.1	1.9	374.6	17.8	4.1
Dictyotopsis propagulifera	59.9	ND	62.5	1.3	0.6
Dilophus okamurae	191.0 (±60.3)	0.3 (±0.3)	206.2 (±28.4)	20.2 (±7.7)	7.1 (±5.2)
Distromium decumbens	106.0 (±10.1)	6.9 (±5.2)	151.3 (±17.4)	27.3 (±3.3)	6.8 (±4.5)
Lobophora variegata	134.5 (±12.6)	ND	97.6 (±24.8)	11.9 (±4.0)	2.5 (±0.7)
Pachydictyon coriaceum	232.0 (±87.5)	0.3 (±0.3)	381.0 (±95.8)	44.7 (±14.3)	8.5 (±4.2)
Padina arborescens	169.5 (±41.8)	ND	187.5 (±29.1)	44.2 (±17.2)	6.7 (±3.0)
P. crassa	66.6 (±5.8)	0.9 (±0.5)	137.3 (±16.0)	184.1 (±44.8)	25.0 (±4.8)
P. minor	75.2 (±12.0)	1.5 (±1.0)	98.9 (± 24.1)	159.4 (±37.0)	24.2 (±2.6)
P. sp.	82.4	ND	198.4	64.7	4.2
Spatoglossum crassum[a]	99.6 (±10.3)	3.2 (±0.4)	162.2 (±12.2)	23.7 (±2.2)	21.3 (±1.8)
S. pacificum	110.7 (±8.6)	ND	300.3 (±57.4)	10.7 (±3.9)	2.2 (±0.6)
Stypopodium zonale	49.9 (±10.7)	1.6 (±0.7)	112.0 (±24.4)	184.6 (±32.7)	12.4 (±2.5)
Taonia leunebackerae	72.5	1.2	116.0	31.8	4.4
Zonaria diesingiata	78.1 (±5.0)	0.7 (±0.5)	121.8 (±27.0)	3.3 (±0.8)	1.1 (±0.6)
Desmarestiales					
Desmarestia acleata	73.2 (±1.0)	1.3 (±1.3)	147.0 (±6.2)	0.5 (±0.5)	ND
D. ligulata[a]	299.7 (±84.8)	2.6 (±2.6)	240.4 (±58.6)	68.3 (±18.5)	68.3 (±24.1)
D. tabacoides[a]	177.0 (±16.4)	6.1 (±1.6)	125.0 (±16.5)	32.7 (±3.4)	21.4 (±4.6)
D. viridis[a]	86.2 (±17.9)	1.0 (±0.8)	136.5 (±18.5)	23.6 (±3.7)	30.7 (±6.0)
Seawater	360.1 (±41.3)	ND	7.5 (±0.8)	38.9 (±5.1)	7.8 (±1.2)

[a] Acidic species.

other nonacidic species (ca. 5.5–8.0; *Dictyotopsis propagulifera, Distromium decumbens, Stypopodium zonale,* and *Taonia lennebackerae*). Among these taxa, the *Dictyotopsis* and *Taonia* material was from cultures. Therefore, culture media might affect the ion compositions in the cells, although other culture material (*Padina* sp.) did not show this tendency. Samples of *Distromium* (Cheju Island) and a part of *Stypopodium* (Seto) were not extracted at the collection site, and 1–2 days elapsed during transportation to the laboratory before the extraction. This might have caused some damage to the samples and affected the results.

The main counterion of H^+ in *Desmarestia* species as well as in dictyotalean species was confirmed by ion chromatography to be SO_4^{2-} (Fig. 3, Table 5). About 66%–73% of total anions was SO_4^{2-} in highly acidic species. The concentration of SO_4^{2-} was about 5–10 times higher than seawater in those species (Table 3). These species contained considerably lower concentrations of Cl^- compared to nonacidic species and seawater (Fig. 3, Tables 3, 5). This shows that the mechanism generating the very low intracellular pH is the same in Desmarestiales and Dictyotales. However, some nonacidic species (*Dictyota divaricata, Padina crassa,* and *P. minor*) also contained relatively high concentrations of SO_4^{2-} (>60%) comparable to those of highly acidic species. These species contained relatively high concentrations of Mg^{2+}, and their total anion/cation ratio is thereby balanced. All species examined contained considerably lower concentrations of Na^+ (ca. 25%–50 %) compared to seawater and contained about the same or slightly higher concentration of K^+. Acidic species contained less Na^+ and K^+ compared to nonacidic species.

The present results suggest that the extremely low intracellular pH in Dictyotales is not caused solely by the accumulation of H^+ but also by the accumulation of SO_4^{2-} and Mg^{2+} as counterions. In other words, both *Dictyopteris* and *Spatoglossum,* and perhaps other dictyotalean genera as well, potentially have a tendency to accumulate SO_4^{2-} in the cell, and, by a change of total ion balance, high acidity appears as a phenetic character in some species.

Nevertheless, the occurrence of the phenetic character of high acidity in the two genera *Dictyopteris* and *Spatoglossum* suggests that the classification of these genera should be reexamined. Genera of the order Dictyotales are defined by thallus structure and the number and shape of apical cells. All species of the order Dictyotales are generally treated in a single family: Dictyotaceae. However, they are sometimes classified into two tribes—*Dictyoteae* Lamouroux ex Dumortier and *Zonarieae* De Toni—on the basis of the presence of a single apical cell or

TABLE 3. Extended.

Cl^-	Br^-	NO_3^-	SO_4^{2-}	Σ cations	Σ anions	Σ anions/Σ σcations	Number of samples
184.9	22.3	ND	28.9	256.4	265.2	1.03	2
225.6 (±67.4)	4.1 (±3.9)	ND	197.0 (±32.5)	514.4 (±76.7)	623.6 (±106.3)	1.21	4
138.8 (±20.8)	1.2 (±0.7)	0.3 (±0.2)	211.9 (±8.0)	411.6 (±22.0)	580.9 (±31.6)	1.41	38
182.4	2.4	44.1	277.5	682.5	783.8	1.15	1
338.6 (±35.7)	2.5 (±1.8)	1.1 (±0.6)	19.5 (±4.1)	431.7 (±52.7)	381.1 (±41.9)	0.88	9
251.0 (±14.6)	1.2 (±0.6)	0.7 (±0.5)	105.5 (±13.5)	483.2 (±25.5)	463.9 (±26.0)	0.96	35
95.6	0.8	ND	108.9	336.0	314.2	0.94	2
428.7	20.5	ND	34.3	553.1	517.7	0.94	2
44.4	0.2	20.5	2.5	126.3	70.1	0.56	2
363.1 (±104.8)	2.7 (±1.0)	ND	24.7 (±6.2)	452.3 (±109.1)	415.0 (±110.9)	0.92	5
144.8 (±30.6)	ND	ND	35.2 (±3.7)	332.6 (±36.5)	215.3 (±36.5)	0.65	3
175.6 (±33.1)	0.7 (±0.7)	ND	23.4 (±3.4)	261.0 (±33.9)	223.2 (±39.3)	0.86	3
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
319.7 (±75.7)	5.5 (±3.6)	0.9 (±0.6)	61.0 (±20.8)	458.7 (±98.8)	448.0 (±111.5)	0.98	6
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
110.7 (±10.6)	0.9 (±0.9)	5.7 (±1.8)	193.0 (±24.7)	542.7 (±62.1)	503.4 (±57.1)	0.93	6
248.2	0.4	16.4	49.8	418.5	364.6	0.87	2
122.8 (±10.3)	7.1 (±7.1)	1.6 (±1.3)	237.2 (±34.1)	355.1 (±20.5)	606.0 (±78.6)	1.71	5
346.0 (±65.5)	1.5 (±0.9)	0.6 (±0.4)	22.0 (±1.3)	436.8 (±64.4)	392.3 (±65.1)	0.90	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
68.3	1.0	9.8	49.1	262.1	177.4	0.68	2
122.9 (±24.5)	0.7 (±0.3)	6.3 (±2.1)	16.9 (±2.0)	209.6 (±25.7)	163.7 (±26.2)	0.78	8
212.2 (±142)	0.2 (±0.2)	ND	2.1 (±0.7)	222.4 (±7.6)	216.7 (±12.8)	0.97	4
313.2 (±83.5)	4.1 (±3.0)	ND	349.7 (±112.6)	821.8 (±222.5)	1016.7 (±292.9)	1.24	3
141.4 (±31.1)	ND	0.7 (±0.7)	141.8 (±15.1)	416.2 (±27.0)	425.7 (±43.7)	1.02	4
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
379.6 (±60.2)	0.9 (±0.2)	ND	26.3 (±1.5)	460.9 (±54.7)	433.1 (±61.1)	0.94	7

else a terminal row of apical cells (Womersley 1987). Both of the acidic species in the order are placed in the tribe *Zonarieae*. *Dictyopteris* and *Spatoglossum* have basically the same thallus construction and apical cells but are distinguished mainly by the presence and absence of midribs. However, midribs are not very clear in some species of *Dictyopteris* (e.g. *D. repens*). Therefore, it would be worth reexamining phylogenetic relationships by some other taxonomic criterion, such as molecular phylogeny.

One plausible explanation of the ecological significance of high acidity of certain algal species is avoidance of herbivory. Anderson and Velimirov (1982) showed that acidic *Desmarestia* species (*D. firma* (C. Ag.) Skottsberg) are consumed less by sea urchins compared with other macroalgal species and attributed its low palatability to its high sulfuric acid concentrations. However, abalones and sea urchins are known to show low preference toward dictyotalean species (Taniguchi et al. 1989, Shiraishi et al. 1991, Duffy and Hay 1994). Aplysia (sea hare) showed a lower preference for *Pachydictyon coriaceum* (nonacidic) than to *Dictyopteris prolifera* (acidic) (Nagahama and Shin 1998). In these cases, diterpenoids contained in these species were shown to have feeding deterrent effects for abalone (Kurata et al. 1990). Therefore, the effect of high acidity on avoidance of herbivory might be questioned in these cases and leads to the notion that the concentration of SO_4^{2-} and Mg^{2+} might have some other adaptive features in the Dictyotales. It is noteworthy that species of Dictyotales have a rather broad geographical distribution in tropical and warm-water regions and have an isomorphic life history. Therefore, in contrast to Desmarestiales, which have a heteromorphic life history and occur in temperate to polar seas, the macroscopic thallus of Dictyotales must withstand more severe environmental stresses (e.g. high temperature and salinity changes) and might have evolved the ability to adapt to these environmental

TABLE 4. Comparison of proton concentrations in acidic species, estimated from ion chromatography data (Σ anions − Σ cations) and pH measurement data.

Species	*Dictyopteris latiuscula*	*Dictyopteris prolifera*	*Dictyopteris repens*	*Spatoglossum crassum*	*Desmarestia aculeata*	*Desmarestia tabacoides*	*Desmarestia viridis*
Σ anions − Σ cations (mM)	109.2	169.3	101.3	251.0	194.8	9.4	310.6
Proton concentrations estimated from pH data (mM)	125.9	158.5	158.5	316.2	158.5	158.5	316.2

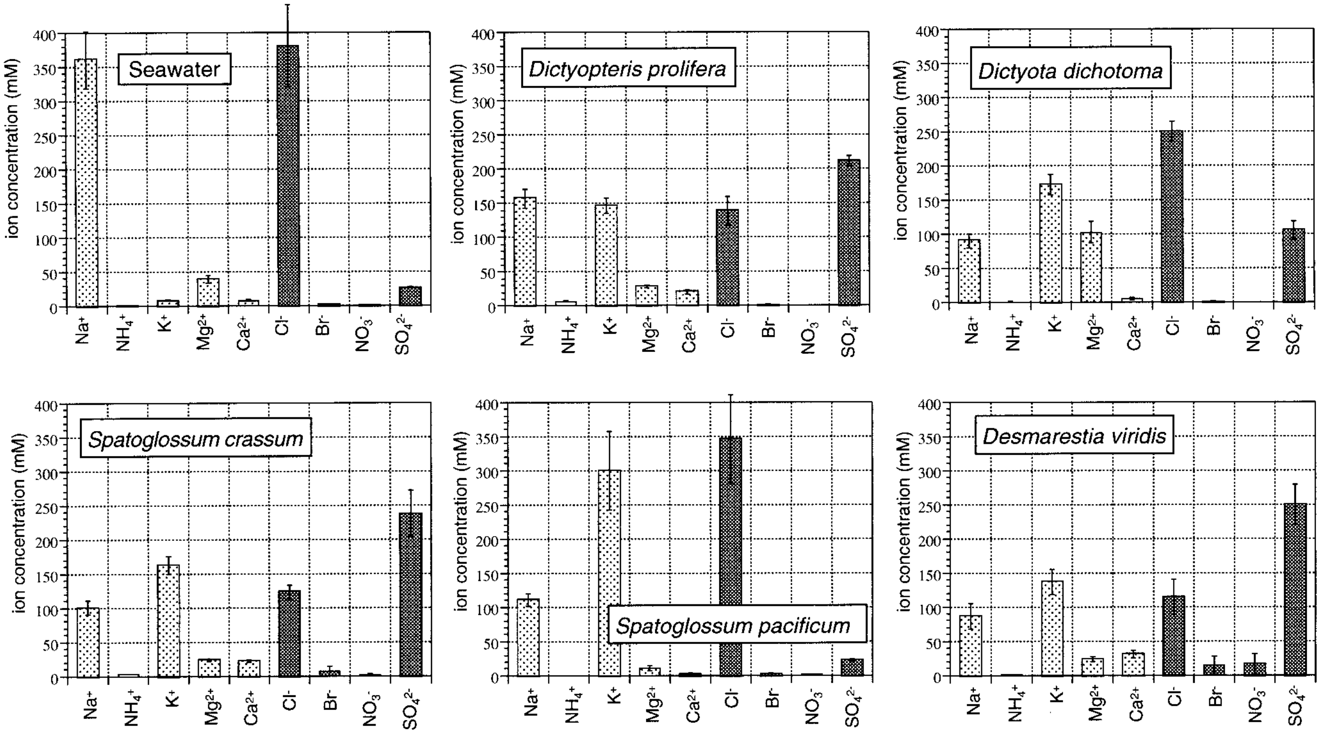

FIG. 3. Intracellular ionic concentrations (estimated from those data of cell extracts) of representative acidic and nonacidic species: *Dictyopteris prolifera, Spatoglossum crassum, Desmarestia viridis* (acidic), *Dictyota dichotoma,* and *Spatoglossum pacificum* (nonacidic). Ionic compositions of seawater (average of seven samples) are also shown. For number of samples in each species and the data for other species, see Table 3.

TABLE 5. Ionic composition (%) in extracts of cations and of anions.

Species	Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	Br^-	NO_3^-	SO_4^{2-}	Number of samples
Dictyotales										
Dictyopteris divaricata	30.2	0.3	64.3	4.5	0.7	69.7	8.4	0.0	21.8	2
Dictyopteris latiuscula[a]	35.2	0.4	36.3	13.7	14.4	36.2	0.6	0.0	63.2	4
Dictyopteris prolifera[a]	38.2	1.6	35.7	14.2	9.9	23.9	0.2	0.1	73.0	38
Dictyopteris repens[a]	23.7	2.4	22.1	16.1	35.7	23.3	0.3	5.6	70.8	1
Dictyopteris undulata	36.1	0.5	52.7	8.3	2.4	88.9	0.7	0.3	10.2	9
Dictyota dichotoma	18.8	0.3	35.9	42.6	2.4	54.1	0.3	0.1	45.5	35
Dictyota divaricata	17.1	0.4	24.7	51.3	6.6	30.4	0.3	0.0	69.3	2
Dictyota linearis	24.1	0.3	67.7	6.4	1.5	82.8	4.0	0.0	13.2	2
Dictyotopsis propagulifera	47.4	0.0	49.5	2.1	1.0	63.3	0.3	29.2	7.2	2
Dilophus okamurae	42.2	0.1	45.6	9.0	3.1	87.5	0.6	0.0	11.9	5
Distromium decumbens	31.9	2.1	45.5	16.4	4.1	67.3	0.0	0.0	32.7	3
Lobophora variegata	51.5	0.0	37.4	9.1	1.9	78.7	0.3	0.0	21.0	3
Pachydictyon coriaceum	32.2	0.0	52.9	12.4	2.4	89.4	0.8	0.1	9.7	4
Padina arborescens	37.0	0.0	40.9	19.3	2.9	71.4	1.2	0.2	27.2	6
Padina crassa	10.7	0.2	22.0	59.1	8.0	31.9	0.1	4.2	63.9	8
Padina minor	13.9	0.3	18.2	58.7	8.9	22.0	0.2	1.1	76.7	6
Padina sp.	19.7	0.0	47.4	30.9	2.0	68.1	0.1	4.5	27.3	2
Spatoglossum crassum[a]	28.1	0.9	45.7	13.4	12.0	20.3	1.2	0.3	78.3	5
Spatoglossum pacificum	25.3	0.0	68.8	4.9	1.0	88.2	0.4	0.2	11.2	5
Stypopodium zonale	8.9	0.3	20.1	66.2	4.5	84.4	0.1	1.1	14.5	6
Taonia leunebackerae	27.7	0.5	44.2	24.3	3.3	38.5	0.5	5.5	55.4	2
Zonaria diesingiata	37.3	0.4	58.1	3.2	1.1	75.1	0.5	3.8	20.6	8
Desmarestiales										
Desmarestia aculeata	32.9	0.6	66.1	0.4	0.0	98.0	0.1	0.0	2.0	4
Desmarestia ligulata[a]	36.5	0.3	29.2	16.6	16.6	30.8	0.4	0.0	68.8	3
Desmarestia tabacoides[a]	42.5	1.5	30.0	15.7	10.3	33.2	0.0	0.2	66.6	4
Desmarestia viridis[a]	25.9	0.3	41.1	14.2	18.5	17.9	2.2	2.5	77.5	4
Seawater	78.1	0.0	1.6	16.9	3.4	87.6	0.2	0.0	12.2	7

[a] Acidic species.

conditions by changing the ionic composition of their cells.

Sulfate was suggested to be localized in the vacuoles of desmarestialean cells (Eppley and Bovell 1958). To determine where high concentrations of SO_4^{2-} were localized within cells, Neutral Red, Brilliant Cresyl Blue, and Quinacrine (a fluorescent dye) were used as vital stains (data not shown). However, the staining was not specific enough to determine the localization of any low-pH sites, perhaps because of the extremely low pH as well as the presence of intense autofluorescence from chloroplasts.

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