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Short Paper

Distribution of free L-cysteine and glutathione in seaweeds

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Aqueous ethanolic extracts of seaweeds are generally rich in alanine, glutamic acid, and their amides but are deficient in the basic amino acids, tryptophan, methionine, and cystine.[1] Amano *et al.*,[2] however, recently found that free-cysteine is accumulated in the cell sap of the giant coenocytic green algae *Valonia macrophysa* and *Ventricaria ventricosa* along with small amounts of glutathione (GSH). In the present study, the distribution of free-cysteine and GSH was investigated for a wide range of macroalgae. The aim was to determine whether *V. macrophysa* and *V. ventricosa* are unique among seaweeds from the standpoint of cysteine accumulation.

Thirty-seven species of algae including nine Chlorophyta, 16 Phaeophyta and 12 Rhodophyta were collected from the coast of Mie Prefecture and transported to the Laboratory of Marine Biochemistry (Mie University, Tsu, Japan) in cooled seawater. A known weight of algal tissue was homogenized in a mortar with 10 volumes of 0.1 M sodium acetate-HCl buffer (pH 2.0) containing 1 mM EDTA. After centrifugation at 34 000 *g* for 15 min, supernatant was pipetted off and collected. The amounts of cysteine and GSH contained in the supernatant were measured by a fluorometric detection method employing pre-column derivatization with the thiol-specific reagent, *N*-(7-dimethylamino-4-methylcoumarinyl) maleimide (DACM), as described previously.[2] Briefly, 20 μL of DACM (1 mmol/L acetone) was added to 1 mL of the supernatant and the pH of the solution was adjusted to 8.6 with 1 M NaOH. The amounts of cysteine and GSH were analyzed by HPLC (Shimadzu LC-9 A; Shimadzu, Kyoto, Japan) equipped with a gel filtration column (Asahi pack GS-320 M, 7.6 × 100 mm; Shimadzu GLC Center, Osaka, Japan) and Shimadzu RF-550 fluorescence HPLC monitor (Shimadzu). A mobile phase was 50 mM Tris-HCl buffer (pH 7.2) containing 0.1 M KCl. The recoveries of cysteine and GSH by this method were almost 90%, regardless of algal species (data not shown).

Table 1 shows the amounts of cysteine and GSH in 37 algal species. Cysteine was only detected in *V. macrophysa* and *V. ventricosa*, at levels of 55 mg/100 g and 16 mg/100 g, respectively. In all other species, cysteine was not detected. Similar results were obtained by the post-column technique using reverse phase column (Develosil ODS-UG-5, 6.0 × 250 mm; Nomura Chemical Co. Ltd, Seto, Japan) and *N*-(9-acridinyl) maleimide as a fluorescent reagent essentially according to Ueda *et al.*[3]

In contrast, contents of GSH in algae widely ranged. In the Chlorophyta, the amounts of GSH were very low in *V. macrophysa* (6 mg/100 g) and *V. ventricosa* (2 mg/100 g). It was noted that the content of cysteine was 9.2 times higher than that of GSH in *V. macrophysa* and eight times higher in *V. ventricosa*. In the case of Phaeophyta, the amounts of GSH ranged from 19 mg/100 g to 3082 mg/100 g. *Ishige okamurai* and *Sargassum thunbergii* had exceptionally high levels of GSH. For example, the amount of GSH in *I. okamurai* was 162 times higher than levels found in *Colpomenia sinuosa* and *Hizikia fusiformis* which had the lowest contents of GSH. In the Rhodophyta, GSH contents ranged from 0.1 mg/100 g to 200 mg/100 g. It should be noted that since *Corallina pilulifera* belongs to the family Corallinaceae, the very low GSH content (0.1 mg/100 g) found in this alga is probably an artifact of the high calcium carbonate content of the cell wall. It is expected that this markedly decreased the relative amounts of 'tissue' analyzed in the 100 g weight of thallus.

It can be concluded from this study that *V. macrophysa* and *V. ventricosa* are indeed unique

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Table 1 Cysteine and glutathione contents of seaweeds (mg/100 g dry weight)

Algae	Cysteine	Glutathione
Chlorophyta		
Hitoegusa (*Monostroma nitidum*)	ND*	49
Anaaosa (*Ulva pertusa*)	ND	113
Usubaaonori (*Enteromorpha linza*)	ND	43
Tamagobaronia (*Valonia macrophysa*)	55	6
Oobaronia (*Ventricaria ventricosa*)	16	2
Hosojuzumo (*Chaetomorpha crassa*)	ND	88
Bouaonori (*C. intestinalis*)	ND	21
Miru (*Codium fragile*)	ND	112
Nagamiru (*C. cylindricum*)	ND	99
Phaeophyta		
Umiuchiwa (*Padina arborescens*)	ND	60
Ishige (*Ishige okamurai*)	ND	3082
Iwahige (*Myelophycus caespitosus*)	ND	53
Kayamonori (*Scytosiphon lomentaria*)	ND	48
Fukuronori (*Colpomenia sinuosa*)	ND	19
Kagomenori (*Hydroclathrus clathratus*)	ND	37
Seiyouhabanori (*Petalonia fascia*)	ND	37
Arame (*Eisenia bicyclis*)	ND	20
Kajime (*Ecklonia cava*)	ND	24
Hijiki (*Hizikia fusiformis*)	ND	19
Mametawara (*Sargassum piluliferum*)	ND	47
Yatsumatamoku (*S. patens*)	ND	35
Akamoku (*S. horneri*)	ND	169
Umitoranoo (*S. thunbergii*)	ND	1432
Tamahahakimoku (*S. muticum*)	ND	71
Isomoku (*S. hemiphyllum*)	ND	52
Rhodophyta		
Susabinori (*Porphyra yezoensis*)	ND	51
Makusa (*Gelidium amansii*)	ND	22
Pirihiba (*Corallina pilulifera*)	ND	0.1
Mukadenori (*Grateloupia filicina*)	ND	24
Tanbanori (*G. elliptica*)	ND	98
Tsurutsuru (*G. turuturu*)	ND	39
Hijirimen (*Cyrtymenia sparsa*)	ND	27
Fukurofunori (*Gloiopeltis furcata*)	ND	48
Tsukasaami (*Callymenia perforata*)	ND	200
Ogonori (*Gracilaria verrucosa*)	ND	21
Kabanori (*G. textorii*)	ND	46
Ayanishiki (*Martensia denticulata*)	ND	23

*ND, not detected.

among the macroalgae surveyed in terms of their ability to accumulate cysteine. The high contents of GSH in *I. okamurai* and *S. thunbergii* are also noteworthy. It is known that cysteine and GSH have many bioactive functions including protection of cells against oxidative damage.[4] Under conditions of environmental stress, such as high light levels, high oxygen levels, or the presence of heavy metals and other pollutants, GSH levels increase with the increase in anti-oxidative substances such as ascorbic acid.[5,6] However, the levels of GSH in *V. macrophysa* and *V. ventricosa* were low (2–6 mg/100 g), as were the amounts of ascorbic acid (10–15 mg/100 g; data not shown), relative to all the algae analyzed. It has been reported that GSH functions as a storage pool of excess cysteine, and that cysteine can be supplied by the degradation of GSH during sulfur starvation.[7] However, in this study, it is unlikely that sulfur starvation occurred because seawater contains ample amounts of sulfur. At this time, it is not clear why there are high amounts of cysteine and correspondingly low contents of GSH in *V. macrophysa* and *V. ventricosa*. Further studies are needed to elucidate the significance of the unusual ratio of cysteine and GSH in these algae.

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