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Studies on the Sulfur Uptake by *Porphyra tenera* and *Ulva pertusa*, Using ^{35}S II.

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(Fig. 1; Tables 1-5)

In the previous paper,[1] authors have reported that in *Ulva pertusa* the rate of total sulfur uptake increased rapidly for six to twelve hours from begining of culture, and thereafter, the increase become slow. Moreover, it was observed that the major portion of sulfur assimilated by the fronds of both *Porphyra tenera* and *Ulva pertusa* were found in the fraction extracted with hot water, particularly in ethanol insoluble. When the ethanol soluble was fractionated with ion exchange resin, the most of sulfur existed in acidic fraction. The substances in which radioactive sulfur was incorporated in this fraction were not clarified.

In this paper, we experimented on the changes of sulfur uptake in various fractions of *Porphyra tenera* with time course of culture using ^{35}S for comparing with those of *Ulva pertusa*. In addition, we experimented on the behaviour of ^{35}S incorporated in the substances of cationic resin non-adsorbables, which were fractionated from ethanol soluble fractions, and attempted to clarify the items of them by the techniques of paperchromatography and autoradiography. The same attempts were made for other seaweeds, *Monostroma nitidum* and *Gracilaria vercosa*.

EXPERIMENTAL

The seaweeds used in this work, were collected at the shore of Seto Inland Sea, neighbouring Fukuyama, Hiroshima Prefecture. They were washed thoroughly with 2.5% NaCl solution, cut into pieces of 2~3 cm square, and dehydrated by centrifugation.

The dehydrated fronds were dipped into 2 *l* of seawater in flasks at 13°C, and cultured under the conditions of about 2% CO_2-containing air bubbling through, and illuminated by light of fluorescent lamps of about 6,000 lux. The seawater nourished with 0.1 g of $NaNO_3$, 0.02 g of $Na_2HPO_4 12H_2O$, and 2.5 m*l* of P-1 solution,[2] and added $H_2{}^{35}SO_4$.

Finishing the culture, the fronds were washed thoroughly with distilled water and dried in an oven at 80°C. Then the dried fronds were extracted and fractionated in accordance with the scheme as shown in figure 1.

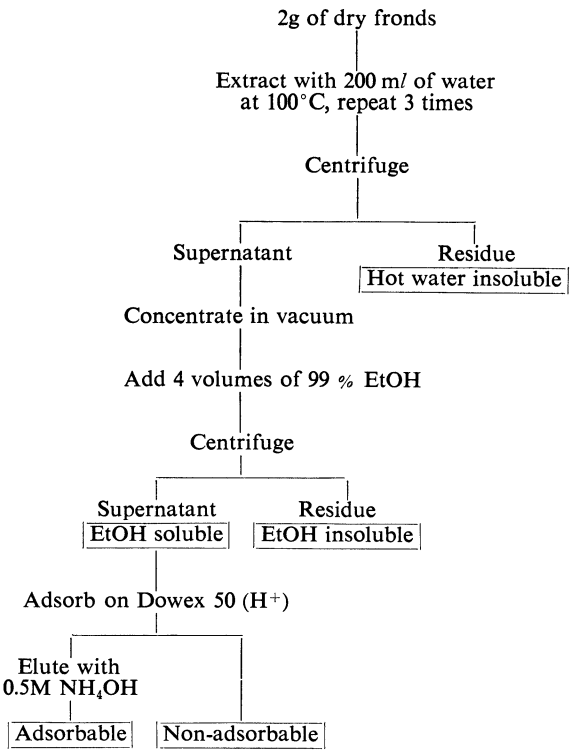

Fig. 1 Procedure of extraction and fractionation of the fronds of *Porphyra tenera*

Sulfur contents of each fractions were determined volumetrically with EDTA after oxydation by Pirie's method,[3] and radioactivities were measured by using a 2π gas flow counter.

Paperchromatography was run on Tôyô-roshi No. 51 (2×40 cm) in two solvent systems, phenol saturated with water and pyridine: acetic acid: water (50: 35: 15), and ninhydrin and *o*-phthalaldehyde[4] were used as color reagents.

The radioactive substances located on the strips were detected by the autoradiograms prepared by means of placing them contact with Fuji no-screen X-ray films for 3 weeks.

RESULTS AND DISCUSSION

Sulfur contents and the changes of ^{35}S activities with time course of culture in *Porphyra tenera* As may be seen in Table 1, a major portion of sulfur is in the ethanol insoluble. These results were the same as previously reported[1].

^{35}S activities in each fractions are shown in Table 2. They were scarcely found in all fractions under darkness, on the contrary, under light they increased progressively. Particularly, it is very remarkable in ethanol insoluble fractions, at 0.5 hours the ^{35}S activity under light was found about 10-fold, and at 24 hours it was

over 30-fold in the case of those under darkness.

Table 1. Sulfur contents of the fronds of *Porphyra tenera*
(mg/g dry frond)

Hot water soluble S		Hot water insoluble S	Total S
EtOH sol. S	EtOH insol. S		
3.0	12.3	4.5	19.9

Table 2. Changes of ^{35}S activities in various fractions of *Porphyra tenera* cultured under light and darkness (cpm $\times 10^3$/g dry frond)

Culture periods (hrs.)	Light					Darkness				
	0.5	2	6	24	48	0.5	2	6	24	48*
Whole frond	23.3	42.6	117.5	455.0	720.8	4.2	5.5	13.3	25.6	521.0
Hot water soluble EtOH soluble	2.7	4.7	16.4	57.8	95.1	1.3	2.0	3.0	8.3	62.0
EtOH insoluble	17.1	25.8	74.3	331.5	486.5	1.8	2.6	5.0	9.4	287.5
Hot water insoluble	1.9	10.1	26.4	60.9	104.4	0.5	1.0	2.2	8.4	138.8

* It was cultured under darkness for 24 hours subsequently under light for 48 hours.

From these observations, under light conditions, it seems reasonable to suppose that the sulfur taken up in the fronds of *Porphyra tenera* turn over progressively. There are points of resemblance in aspects of sulfur uptake in each fractions of both *Pophyra tenera* and *Ulva pertusa*.

^{35}S activities in ethanol solubles are shown in Table 3. The bulk amount of ^{35}S of this fraction was found in non-adsorbable on Dowex 50 (H^+) as similar as the acidic fraction designated in the previous paper.[1)]

Table 3. ^{35}S activities of fractions separated from EtOH solubles with Dowex 50 (H^+)
(cpm $\times 10^3$/g dry frond)

Culture periods (hrs.)	6	48	48*
Adsorbable	0.6	4.7	1.8
Non-adsorbable	13.6	79.7	53.6

* It was cultured under darkness for 24 hours subsequently under light for 48 hours.

Behaviour of ^{35}S in the fractions of non-adsorbables on Dowex 50 (H^+)
An attempt was made to clarify the behaviour of ^{35}S incorporated substances in the fractions of non-adsorbables by the techniques of the paperchromatography and autoradiography. The results are shown in Table 4-1 and -2.

Table 4-1. Color intensities (C.I.) and radioactivities (R.A.) of the substances located on chromatograms*
(*Porphyra*)

Culture periods	10 min.		30 min.		2 hours		24 hours	
	C.I.	R.A.	C.I.	R.A.	C.I.	R.A.	C.I.	R.A.
Spot I	++	−	++	±	++	+	++	++
Spot II	+	−	+	−	+	−	+	±
Spot III	−	−	−	−	−	±	−	+

* These chromatograms were run in phenol saturated with water and colored with ninhydrin.

Table 4-2. R_f values of the substances located on chromatograms
(*Porphyra*)

	R_f		Colors developed with *o*-phthalaldehyde
	A*	B**	
Spot I	0.42	0.38	reddish orange
Spot II	0.23	—	weak grey
Spot III	0.10	0.25	—
Authentic taurine	0.42	0.38	reddish orange
Inorganic SO_4^{--}	0.10	0.25	—

* Run in phenol saturated with water
** Run in pyridine: acetic acid: water (50 : 35 : 15)

It was observed that three radioactive spot I II and III emerged on the autoradiogram of 24 hours. As for the spot I and II, they were also detected by both reagents, *o*-phthalaldehyde and ninhydrin, but spot III was not detected by these reagents. Further, in color intensities of both spot I and II, there were scarcely differences among all chromatograms, on the contrary, there were considerable differences with time course of culture in radioactivities of each spots. The radioactivities of spot I could be slightly detected on autoradiogram of 30 minutes, then increased with time course of culture, and detected strongly in 24 hours. The radioactivity of spot II could be only weakly detected in 24 hours, and those of spot III could be detected 2 and 24 hours.

From co-chromatography with authentic taurine and inorganic radioactive sulfate, it was confirmed that the spot I is taurine and III is inorganic sulfate, but the spot II is yet unknown.

For comparing these results with those of other seaweeds, the fronds of *Monostroma nitidum* and *Gracilaria vercosa* were treated by the same procedures as described above. The results are shown in Table 5-1 and -2.

In *Gracilaria,* two radioactive spot I and II were observed on the autoradiograms of 2 and 24 hours. The spot I were detected with the reagents, both *o*-phthalaldehyde and ninhydrin, on every chromatograms, but the radioactivities of those were strong in 24 hours and weak in 2 hours, and could not be detected in

30 and 10 minutes. The spot II were detected also on every other autoradiograms as well as 24 hours, but they were negative for both reagents.

Table 5-1. Color intensities (C.I.) and radioactivities (R.A.) of the substances located on chromatograms*
(*Gracilaria* and *Monostroma*)

Culture periods	10 min.		30 min.		2 hours		24 hours	
	C.I.	R.A.	C.I.	R.A.	C.I.	R.A.	C.I.	R.A.
Gracilaria								
Spot I	++	−	++	−	++	+	++	++
Spot II	−	+	−	+	−	+	−	+
Monostroma								
Spot I	++	−	++	−	++	+	++	++
Spot II	+	−	+	−	+	−	+	+
Spot III	−	+	−	+	−	+	−	+

* These chromatograms were run in phenol saturated with water and colored with ninhydrin.

Table 5-2. R_f values of the substances located on chromatograms
(*Gracilaria* and *Monostroma*)

	R_f		Colors developed with *o*-phthalaldehyde
	A*	B**	
Gracilaria			
Spot I	0.42	0.38	reddish orange
Spot II	0.10	0.25	—
Monostroma			
Spot I	0.36	0.42	yellow
Spot II	0.25	—	weak grey
Spot III	0.10	0.25	—
Authentic			
taurine	0.42	0.38	reddish orange
D-cystenolic acid	0.36	0.42	yellow
Inorganic SO_4^{--}	0.10	0.25	—

* Run in phenol saturated with water
** Run in pyridine: acetic acid: water (50 : 35 : 15)

In *Monostroma,* there found three radioactive spot I, II and III, on the autoradiograms of 24 hours, and the spot I and II were detected with both color reagents on every chromatograms, and the spot III of those were negative for the color reagents. The changes of radioactivities of spot I and III in the culture periods were similar to those of spot I and II of *Gracilaria.*

It was revealed by the co-chromatography that the spot I was taurine in *Gracilaria,* and D-cysteinolic acid in *Monostroma.* The spot II of *Gracilaria* and III of *Monostroma* were confirmed as inorganic sulfate.

From these results, it was confirmed that the large portion of sulfur-containing substances in the non-adsorbables are taurine in *Porphyra* and *Gracilaria,* and D-cysteinolic acid in *Monostroma.* Moreover, radioactive sulfur is actively incorporated in these substances. It would be supposed that in *Porphyra* no appearance of spots at R_f 0.62 and 0.36 were due to less quantity of these substances which had been observed in the previous study.[1)]

These results differ from the data obtained with blue green algae, such as *Chlorella*[5)] and *Euglena,*[6)] in which the most sulfur in ethanol extracts are mainly incorporated into cysteine or cystine and glutathione.

Though in animal tissues, taurine is generally recognized as a metabolic end-product from sulfur-containing amino acids; in these marine algae, it seemed that taurine and D-cysteinolic acid have some important roles in sulfur metabolism.

SUMMARY

1. Sulfur contents of each fractions (ethanol soluble and insoluble, and hot water insoluble) of *Porphyra tenera* were almost stationary throughout the culture.

2. Aspects of sulfur uptake in various fractions of *Porphyra tenera* were as like as those of *Ulva pertusa*[1)].

3. Under light, sulfur uptake by *Porphyra* was very active, but under darkness it was very dull, particularly in ethanol insoluble fraction.

4. In *Porphyra* and *Gracilaria,* a considerable amount of ^{35}S of the non-adsorbable was incorporated into taurine, while in *Monostroma* it was incorporated into D-cysteinolic acid.

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^{35}Sによるアサクサノリおよびアオサの硫黄の吸収に関する研究 II.

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^{35}Sを添加した海水中でアサクサノリを培養し，その葉体を Fig. 1 のように分割し，各フラクションへの ^{35}S の吸収を明暗両条件下で経時的に観察した．

1） 各フラクションの硫黄含量は培養期間（24～48時間）中，ほとんど一定であった．

2） ^{35}S の吸収は明条件下ではきわめて活発で，暗黒条件下では著しくにぶい．また明条件下の各フラクションの吸収状態は前報[1)] アオサの場合とほぼ同様の傾向を示した．

3) アルコール可溶部中の ^{35}S は大部分が陽イオン交換樹脂非吸着性の物質中に移った.

4) 陽イオン交換樹脂非吸着性物質のうちで ^{35}S の存在を最も強く示しているものはタウリンであって，そのほか少量の未確認の物質にもみられ，また $^{35}SO_4^{--}$ のままのものも少量存在した.

5) 陽イオン交換樹脂非吸着性物質における ^{35}S の所在について，ヒトエグサとオゴノリで実験した結果，ヒトエグサでは D- システノール酸に，オゴノリではアサクサノリと同じくタウリンに最も強く認められた.