Isolation of L-Methionine-*l*-sulfoxide and N-Methylmethionine Sulfoxide from a Red Alga *Grateloupia turuturu*

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In the methanolic extract from a red alga *Grateloupia turuturu*, L-methionine-*l*-sulfoxide, an unknown base and glutamic acid were predominant among free amino acids. On acid hydrolysis, the first two compounds disappeared and arginine, citrulline, ornithine and methionine appeared or increased concomitantly. Two methionine derivatives were then isolated from the extract by ion-exchange column chromatography, and identified as L-methionine-*l*-sulfoxide and N-methylmethionine sulfoxide by synthesis. The former sulfoxide was confirmed to be the origin of methionine appearing on acid hydrolysis.

The methanolic extract from a red alga *Grateloupia turuturu* revealed two large peaks of unknown compounds, one appearing before aspartic acid and the other between ammonia and arginine, when analyzed with an automatic amino acid analyzer. On acid hydrolysis, these two compounds disappeared and afforded arginine, citrulline, ornithine and methionine.

One of the unknown compounds appearing just before aspartic acid was isolated by column chromatography using ion-exchange resins as hexagonal plates, and identified as L-methionine-*l*-sulfoxide by synthesis. In addition, we noticed the presence of a large amount of another methionine derivative giving a very weak coloration with ninhydrin reagent in the extract. It was isolated as prisms and identified as N-methylmethionine sulfoxide. This may be the first time that the former sulfoxide was isolated from marine algae and the latter from the biological origin.

The present paper deals with amino acid composition of the extract of *G. turuturu* before and after acid hydrolysis and identification of these two methionine sulfoxides.

Experimental and Results

Material The material of *G. turuturu* was collected at the intertidal region of Hiroshima Bay in winter in 1972 and 1973, and put into methanol immediately after collection.

Amino acid composition One hundred g of the fresh algal sample was kept in 400 ml of methanol for 4 days at room temperature on occasional stirring. The methanolic extract was condensed under reduced pressure in order to remove methanol and defatted with diethyl ether. The aqueous solution was made up to 100 ml and

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used for estimation of amino acids with a Hitachi liquid chromatography 034 type apparatus. A part of the solution was hydrolyzed with an equal volume of conc. HCl in a sealed tube at 110°C for 15 hr. After excess HCl was removed by evaporation, the residue was dissolved in water and applied to the amino acid analyzer.

Table 1. Composition of amino acids in the methanolic extracts of *G. turuturu* (mg in 100 g of fresh alga)

Amino acids	Before hydrolysis		After hydrolysis	
	Concn.	N	Concn.	N
Alanine	4.1	0.65	4.2	0.66
Arginine	trace	—	72.3	23.2
Aspartic acid	4.1	0.43	6.8	0.72
Citrulline	16.0	3.84	37.3	8.95
Glutamic acid	45.0	4.28	98.0	9.32
Glutamine	23.1	4.43	—	—
Glycine	1.5	0.28	6.5	1.21
Histidine	—	—	trace	—
Isoleucine	trace	—	trace	—
Leucine	trace	—	trace	—
Methionine	—	—	41.4	3.89
L-Methionine- <i>l</i> -sulfoxide	68.5	5.81	trace	—
Ornithine	trace	—	14.7	3.12
Phenylalanine	trace	—	trace	—
Serine	3.6	0.48	8.6	1.15
Unknown base*	58.9	18.9	trace	—
Valine	trace	—	trace	—
NH ₃		0.49		8.31
Total		39.6		60.5
Total N of the extract				98.0

* Calculated as arginine.

The amino acid compositions before and after acid hydrolysis are shown in Table 1. Free amino acid nitrogen accounts for 40% of total nitrogen of the extract before hydrolysis and 62% after hydrolysis. The unknown base appearing as a large broad peak between ammonia and arginine is calculated as arginine. The unknown base, L-methionine-*l*-sulfoxide, glutamic acid and citrulline, especially the first two, are predominant. After hydrolysis, the unknown base, L-methionine-*l*-sulfoxide and glutamine disappear almost completely and are replaced by arginine, citrulline, glutamic acid, methionine and ornithine. As described below, methionine was confirmed to be derived from L-methionine-*l*-sulfoxide. The increase of arginine, citrulline and ornithine suggests that the unknown base is a peptide containing the arginine and citrulline moieties.

Separation of amino acids Ten kg of fresh algal sample was extracted with 20 liters of methanol and the extract was treated as described above. The clear aqueous solution

obtained was poured onto a column (5×100 cm) of Dowex 50W-X8 (H^+ form, 20-50 mesh). From the column rinsed with water, the adsorbed substances were eluted with $2\ N\ NH_4OH$. The fractions containing ninhydrin-positive substances were collected and concentrated to remove excess ammonia. The resulting solution was then passed through a column of Dowex 50W-X8 (NH_4^+ form). After washing the column with water, the adsorbed basic compounds were eluted with $2\ N\ NH_4OH$ and set aside for separation of the unknown base. The effluent and washings were combined, adjusted with dil. HCl to pH 7.0, and applied to a column of Dowex 1-X8 (acetate form) to remove acidic amino acids. The effluent was poured onto a column (2×100 cm) of Dowex 50W-X4 (H^+ form, 100-200 mesh). From the column washed with water, amino acids were eluted with $0.15\ N\ NH_4OH$ and the eluate was collected in 10 ml fractions. Amino acid in each fraction was checked by paper chromatography using Toyo-roshi No. 50 and a mixture of 1-butanol, acetic acid and water (4:1:2, v/v). The spots were visualized with ninhydrin reagent. The substance showing a faint color with ninhydrin reagent appeared as a single spot at R_f 0.45 in the initial 400 ml portion. In the subsequent 300 ml portion, a compound appearing as a clear purple spot at R_f 0.35 was eluted, preceding to citrulline and alanine.

Isolation and identification of L-methionine-*l*-sulfoxide The fractions containing a substance of R_f 0.35 gave colorless crystals when the condensed aqueous solution was mixed with 2 volumes of ethanol and left stand in an ice-box. Recrystallization from aqueous ethanol gave hexagonal plates; m. p. $234^\circ C$ (decompn.) in a sealed tube. Yield 3 g. The molecular weight determined by the cryoscopic method was 163. *Anal.* Found: C, 36.18; H, 6.59; N, 8.22%. Calcd. for $C_5H_{11}NO_3S$: C, 36.35; H, 6.71; N, 8.48% and M.W., 165.2. $[\alpha]_D^{18} = -75^\circ$ ($c=1$ in water). Nitrogen was found to be amino-N by the Van Slyke method. It developed the purple, red and yellow colors with isatin, diazo and Nessler's reagents, respectively, and was positive to sodium nitroprusside after fusion with metallic sodium, indicating the presence of sulfur. Since it liberated iodine from hydriodic acid, sulfur was confirmed to be present as sulfoxide.¹⁾ When heated in $6\ N\ HCl$ at $110^\circ C$ for 18 hr, it afforded methionine that was identified as such by the IR spectrum. Methionine was also obtained by reduction with sodium metabisulfite at room temperature for 2 weeks or with hydriodic acid at $110^\circ C$ in a sealed tube for 18 hr. In oxidation with excess hydrogen peroxide, methionine sulfone was obtained and identified by the IR spectrum. These results clearly indicated that the isolated compound is methionine sulfoxide.

For comparison, L-methionine-*l*-sulfoxide was prepared by the method of LAVINE²⁾ as follows. L-Methionine was oxidized with a small excess of hydrogen peroxide at room temperature for 3 hr. The resulting diastereoisomers, L-methionine-*dl*-sulfoxide, were resolved into *d*- and *l*-forms as picrate. After decomposition of picrate of *l*-form

with a small excess of amylamine, *L*-methionine-*l*-sulfoxide was precipitated with acetone. Recrystallization from aqueous ethanol gave colorless hexagonal plates; m.p. 231–234°C (decompn.) in a sealed tube. $[\alpha]_D^{18} = -57^\circ$ ($c=1$ in water). Ref.²⁾ $[\alpha]_D^{25} = -71.6^\circ$. The value for specific rotation was different from that of the isolated specimen, probably due to incomplete resolution of diastereoisomers. The synthesized specimen, however, was identical with the isolated one in paper chromatography using different solvent systems and in the IR spectrum as shown in Fig. 1. The figure shows a characteristic peak at 1020 cm^{-1} .

Fig. 1. IR spectra of isolated (A) and synthesized (B) *L*-methionine-*l*-sulfoxide (Nujol).

Isolation and identification of *N*-methylmethionine sulfoxide The fractions revealing only one spot at R_f 0.45 were combined. The solution gave colorless crystals when left stand in an ice-box on addition of ethanol. Recrystallization from aqueous ethanol gave prisms; m.p. 226°C (decompn.) in a sealed tube. The yield was approximately 4 g and close to that of *L*-methionine-*l*-sulfoxide. The molecular weight determined by the cryoscopic method was 174. *Anal.* Found: C, 39.97; H, 7.17; N, 7.61%. Calcd. for $C_6H_{13}NO_3S$: C, 40.22; H, 7.31; N, 7.82% and M.W., 179.2. $[\alpha]_D^{18} = -56^\circ$ ($c=1$ in water). This compound gave a weak purple color with ninhydrin, weak blue one with isatin, distinct blue one with sodium nitroprusside and acetaldehyde, and red one with diazo reagents. It evolved no nitrogen gas when treated with nitrous acid. These results indicated the presence of an alkylated amino group. It showed the same results in qualitative tests for sulfur as *L*-methionine-*l*-sulfoxide. A desulfurization product with Raney nickel was *N*-methyl- α -aminobutyric acid. In reduction with sodium metabi-

sulfite or hydriodic acid, it gave a product identical with *N*-methylmethionine prepared from methionine by the method of IZUMIYA.³⁾ These degradation products were identified by paper chromatography as shown in Table 2.

Table 2. Rf values of *N*-methylmethionine sulfoxide and its related compounds

Solvents	A	B	C	D	E
Isolated <i>N</i> -methylmethionine sulfoxide	0.39	0.34	0.07	0.20	0.32
Synthesized <i>N</i> -methylmethionine sulfoxide	0.39	0.34	0.07	0.20	0.32
Reduction product with HI	0.61	0.59	0.35	0.46	0.64
Reduction product with Na ₂ S ₂ O ₅	0.61	0.59	0.35	0.47	0.63
<i>N</i> -Methylmethionine	0.61	0.59	0.35	0.47	0.64
Desulfurization product with Raney nickel	0.52	0.51	0.21	0.28	0.62
<i>N</i> -Methyl- α -aminobutyric acid	0.52	0.51	0.22	0.28	0.62

A, 1-butanol-acetic acid-water (4:1:2). B, 1-butanol-acetic acid-pyridine-water (4:1:1:2). C, 1-butanol-pyridine-water (1:1:1). D, lutidine-collidine-water (1:1:1). E, ethanol-28% ammonia water-water (90:5:5).

When 200 mg of this compound was oxidized with potassium permanganate in acidic solution, 90 mg of colorless needles showing m.p. 244°C (decompn.) was obtained by ion-exchange column chromatography using Dowex 50W-X8 (H⁺ form) and 2 N NH₄OH as eluting solvent. *Anal.* Found: C, 36.86; H, 6.64; N, 7.28%. Calcd. for C₆H₁₃NO₄S: C, 36.91; H, 6.71; N, 7.17%. The IR spectrum coincided well with that of synthesized *N*-methylmethionine sulfone. The same product was also obtained by oxidation with hydrogen peroxide.

For comparison, *N*-methylmethionine sulfoxide was synthesized as follows. *N*-Methylmethionine prepared by the method of IZUMIYA²⁾ was derived to sulfoxide after the method adopted for preparation of methionine sulfoxide. The synthesized specimen was quite identical with the isolated one in paper chromatography as shown in Table 2, although slightly different in the IR spectrum probably due to racemization of the synthesized specimen. From these results, it may be reasonably concluded that this compound is *N*-methylmethionine sulfoxide. The steric configuration still remains undetermined.

Discussion

The occurrence of *L*-methionine-*l*-sulfoxide and *N*-methylmethionine sulfoxide in the methanolic extract of *G. turuturu* was thus established. They seem to reflect the peculiar metabolic pathway of methionine in this alga, and their physiological significance may be an interesting biochemical problem.

In 1964, TAKAGI and OKUMURA⁴⁾ isolated a new amino acid from a red alga *Chondrus ocellatus* and postulated it to be *S*-hydroxymethyl-*L*-homocysteine, an isomer

of methionine sulfoxide. Their specimen coincides well with our specimen in m.p., rotation, IR and color reactions. They excluded the sulfoxide structure, assuming the positive reactions to both diazo and Nessler's reagents and a strong absorption at 1020 cm^{-1} in the IR spectrum as signs of hydroxyl group. We confirmed however that methionine and its sulfoxide are also positive to diazo and Nessler's reagents. In addition, the sulfoxide group is known to have an intense absorption between $1000\text{--}1100\text{ cm}^{-1}$ in the IR spectrum.⁵⁾ The structure of the compound assumed to be *S*-hydroxymethyl-L-homocysteine should be therefore re-examined.

The composition of free amino acids in the extract showed a characteristic pattern and a marked change during acid hydrolysis. Methionine was proved to originate from its sulfoxide. We have recently succeeded in isolating a new dipeptide as the origin of arginine, citrulline and ornithine, as will be reported in the next paper.

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