CHEMICAL STUDIES ON MARINE ALGAE

X. Free and Combined Amino Acids in Marine Algae

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Although only small amounts of nitrate, peptide and organic bases are contained in the protein-free extract of marine algae, it may be considered that the greater part of the extract occurs in the form of free amino acid. Marine algae are characterized by the nitrogen forms in which free amino acids are found to a comparatively rich degree. Ogino[1] has investigated free amino acids occurring in various species of marine algae and recognized, as a rule, the presence of glutamic acid, aspartic acid, alanine, cystine, and arginine. Coulson[2] has also examined free amino acids found in nine species of brown and red marine algae. He reported that glutamic acid, aspartic acid, glycine, alanine, valine, leucine and proline are present in the free state in all the marine algae, whereas cystine, serine, threonine, phenylalanine, arginine and lysine are absent in some species of marine algae, and tyrosine, histidine are absolutely lacking in all species of marine algae examined.

On the other hand, Lugg[3] has investigated the difference in the constituent amino acids of the tissue protein in one marine alga, in four species of cryptogams, *Ulva*, *Lunularia*, *Pteridium* and *Selaginella*, in five species of phanerogams, namely *Dactylis*, *Lolium*, *Beta*, and in two species of *Trifolium*. In addition to the above mentioned experiments in order to ascertain whether there is any correlation between the chemical components among certain of the marine algae and the position occupied by them in the evolutional series, Mazur & Clarke[4] have also determined the amino acids contained in the hot formic acid soluble fractions of (a) *Ulva lactuca*, a green alga, (b) *Laminaria*, and (c) *Sargassum*, both belonging to the brown algae, and (d) *Chondrus crispus* belonging to the red algae.

In the present study, the distribution of the free and combined amino acids occurring in 32 species of marine algae were investigated qualitatively by means of two-dimensional paper chromatography.

Experimental

Immediately after harvesting at Nanaehama and Moheji, both in the vicinity of Hakodate, the fresh marine algae were washed repeatedly with distilled water, dried at room temperature and at 100°C for thirty minutes by the drier, and then milled. Dried materials obtained from the market were also used. Small quantities of these milled materials were extracted three times with 70 per cent ethanol. The insoluble residue, containing 3–5 per cent nitrogen, was then hydrolysed for 24 hrs at 105°C with 6 N

Table 1 (A). Free amino acids

+++, strong ninhydrin colour;
+, weak ninhydrin colour ;

	Ulva pertusa (May 8)	*Enteromorpha Linza* (Mar. 9)	*Enteromorpha Linza* var. *crispata* (May 2)
Cystine	−	+	+
Aspartic acid	++	+	+
Glutamic acid	++	++	++
Serine	+	++	++
Glycine + Threonine	+	++	++
Alanine	++	++	++
Tyrosine	−	−	−
Valine	+	+	−
Methionine	−	−	−
Phenylalanine	−	−	−
Leucine	−	−	−
Proline	++	++	+
Tryptophan	+	+	−
Arginine	++	+	−
Lysine	−	−	−
Histidine	−	−	−

	Chorda Filum (June 14)	*Laminaria japonica* (Apr. 25)	*Undaria pinnatifida* f. *distans* (Apr. 28)
Cystine	−	−	−
Aspartic acid	+++	+	+
Glutamic acid	+++	+++	++
Serine	+	+	−
Glycine + Threonine	++	+	+
Alanine	++	+++	+
Tyrosine	−	−	−
Valine	−	−	−
Methionine	−	−	−
Phenylalanine	−	−	−
Leucine	−	−	−
Proline	++	++	+
Tryptophan	+	−	−
Arginine	+	+	−
Lysine	−	−	−
Histidine	−	−	−

in various species of marine algae

++, moderately strong ninhydrin colour;
−, not detected

Enteromorpha intestinalis (June 14)	*Chaetomorpha moniligera* (June 25)	*Leathesia difformis* (June 14)	*Desmerestia ligulata* (May 2)	*Scytosiphon Lomentaria* (May 2)
+	−	+	+	−
+	++	+	+	++
+	+++	+	+	++
+	+	+	+	++
+	+++	+	+	+++
++	++	++	+	++
−	−	−	−	−
−	+	−	−	+
−	−	−	−	−
−	−	−	−	−
−	−	−	−	−
++	++	++	++	++
−	+	−	−	+
−	+	−	−	+
−	−	−	−	−
−	−	−	−	−

Undaria pinnatifida f. *narutensis*	*Hijikia fusiformis* (June 14)	*Sargassum sagamianum* (June 14)	*Sargassum confusum* (June 14)	*Sargassum Thunbergii* (June 14)
−	−	−	−	−
+	++	++	++	++
+	++	++	++	++
−	−	+	+	+
+	−	+	++	+
+	+	++	+	++
−	−	−	−	−
−	++	−	−	+
−	−	−	−	−
−	−	−	−	−
−	−	−	−	−
+	++	++	++	++
−	−	−	−	−
−	−	−	−	−
−	−	−	−	−
−	−	−	−	−

Table 1 (B). Free amino acids

+++, strong ninhydrin colour;
+, weak ninhydrin colour ;

	Porphyra Okamurai	*Porphyra crispata*	*Porphyra tenera*
Cystine	−	−	−
Aspartic acid	++	++	+++
Glutamic acid	+++	++	+++
Serine	+	+	+
Glycine + Threonine	++	+	+++
Alanine	+++	++	+++
Tyrosine	+	+	+
Valine	−	−	−
Methionine	−	−	−
Phenylalanine	−	−	−
Leucine	−	−	−
Proline	+	+	++
Tryptophan	+	+	+
Arginine	++	+	+
Lysine	−	−	−
Histidine	−	−	−

	Cirrulicalis Gmelini (Feb. 1)	*Gracilaria verrucosa* (June 14)	*Iridophycus laminarioides* (Apr. 24)
Cystine	−	+	−
Aspartic acid	++	++	++
Glutamic acid	++	+	+
Serine	++	++	+
Glycine + Threonine	++	++	++
Alanine	+	++	++
Tyrosine	+	−	−
Valine	−	−	−
Methionine	−	−	−
Phenylalanine	−	−	−
Leucine	−	−	−
Proline	++	++	++
Tryptophan	+	+	+
Arginine	+	+	+
Lysine	−	−	−
Histidine	−	−	−

in various species of marine algae

++, moderately strong ninhydrin colour;
−, not detected

Porphyra pseudolinearis	*Porphyra umbilicalis*	*Neodilsea Yendoana* (June 14)	*Grateloupia filicina* (Feb. 1)	*Grateloupia livida* (Feb. 1)
−	−	−	−	−
++	++	++	++	++
++	+++	++	+	+++
+	+	++	++	+
++	+	+	++	++
++	++	++	++	+++
+	+	+	+	+
−	−	−	+	+
−	−	−	−	−
−	−	−	−	−
−	+	+	−	−
+	+	++	++	++
+	+	+	+	+
+	+	+	+	++
−	−	−	−	−
−	−	−	−	−

Rhodymenia palmata (Apr. 24)	*Lomentaria catenata* (Feb. 1)	*Delesseria violacea* (May 2)	*Polysiphonia senticulosa* (May 2)	*Chondria crassicaulis* (Feb. 1)
−	−	−	+	−
+++	++	++	++	++
+++	+++	+++	+++	++
++	++	+	++	+
+	+	+	++	+
++	+	++	++	++
+	−	−	−	−
−	+	−	−	+
−	−	−	−	−
−	−	−	−	−
−	−	−	−	−
++	++	++	++	++
+	+	+	−	+
+	+	+	+	+
−	−	−	−	−
−	−	−	−	−

Table 2 (A). Combined amino acids

+++, strong ninhydrin colour;
+, weak ninhydrin colour ;

	Ulva pertusa (May 8)	*Enteromorpha Linza* (Mar. 9)	*Enteromorpha Linza* var. *crispata* (May 2)
Cystine	++	++	+++
Aspartic acid	++	++	+
Glutamic acid	+++	++	+
Serine	++	++	++
Glycine + Threonine	++	++	++
Alanine	+++	+++	+++
Tyrosine	−	−	−
Valine	++	++	++
Methionine	+	+	+
Phenylalanine	++	++	+
Leucine	++	+	++
Proline	+	+	+
Tryptophan	+	+	+
Arginine	++	++	++
Lysine	+	+	+
Histidine	+	+	+

	Chorda Filum (June 14)	*Laminaria japonica* (Apr. 25)	*Undaria pinnatifida* f. *distans* (Apr. 28)
Cystine	+	+	+
Aspartic acid	++	+	+
Glutamic acid	++	+	+
Serine	+++	++	+
Glycine + Threonine	+++	++	+++
Alanine	++	++	++
Tyrosine	−	−	−
Valine	++	++	++
Methionine	+	−	+
Phenylalanine	+	++	+++
Leucine	++	++	++
Proline	+	+	+
Tryptophan	+	+	+
Arginine	++	++	++
Lysine	+	+	−
Histidine	+	+	+

in various species of marine algae

++, moderately strong ninhydrin colour;
−, not detected

Enteromorpha intestinalis (June 14)	*Chaetomorpha moniligera* (June 25)	*Leathesia difformis* (June 14)	*Desmerestia ligulata* (May 2)	*Scytosiphon Lomentaria* (May 2)
+	+	++	+	+
+	++	++	++	++
++	+	++	++	+
++	++	+	++	++
+	++	+	++	++
++	++	++	++	++
−	−	−	−	+
++	++	++	++	++
+	+	−	+	+
++	++	+	++	++
+	++	++	++	++
+	+	+	+	+
+	+	+	+	+
++	++	++	++	++
+	+	+	+	+
+	+	+	+	+

Undaria pinnatifida f. *narutensis*	*Hijikia fusiformis* (June 14)	*Sargassum sagamianum* (June 14)	*Sargassum confusum* (June 14)	*Sargassum Thunbergii* (June 14)
+	+	+	+	++
+	+	+	++	++
+	+	+	+	+
++	+	++	++	+
++	++	+++	++	++
++	++	++	++	++
+	−	+	−	−
++	+	++	++	++
+	+	+	+	+
++	++	++	++	++
++	++	++	++	++
+	+	+	+	+
+	+	+	+	+
++	++	++	++	+
+	−	+	+	+
+	−	+	+	−

Table 2 (B). Combined amino acids

+++, strong ninhydrin colour;
+, weak ninhydrin colour;

	Porphyra Okamurai	*Porphyra crispata*	*Porphyra tenera*
Cystine	+	++	+
Aspartic acid	++	++	++
Glutamic acid	+++	++	++
Serine	++	++	++
Glycine + Threonine	++	++	+++
Alanine	+	++	+++
Tyrosine	+	+	+
Valine	++	++	++
Methionine	+	+	+
Phenylalanine	+	++	++
Leucine	++	+++	+++
Proline	+	+	+
Tryptophan	+	+	+
Arginine	++	++	++
Lysine	+	+	+
Histidine	+	+	+

	Cirrulicalis Gmelini (Feb. 1)	*Gracilaria verrucosa* (June 14)	*Iridophycus laminarioides* (Apr. 24)
Cystine	+	+	+
Aspartic acid	++	+	++
Glutamic acid	++	+	+
Serine	++	+	++
Glycine + Threonine	++	++	+++
Alanine	++	+	++
Tyrosine	+	−	−
Valine	++	++	+++
Methionine	+	+	+
Phenylalanine	++	++	++
Leucine	++	++	++
Proline	+	+	+
Tryptophan	+	+	+
Arginine	++	++	++
Lysine	+	+	+
Histidine	+	−	+

in various species of marine algae

++, moderately strong ninhydrin colour;
−, not detected

Porphyra pseudolinearis	*Porphyra umbilicalis*	*Neodilsea Yendoana* (June 14)	*Grateloupia filicina* (Feb. 1)	*Grateloupia livida* (Feb. 1)
+	+	+	+	+
++	++	+	++	+
++	++	+	++	+
+	++	+	++	++
+++	+++	++	++	++
++	++	++	++	++
+	+	+	+	+
++	+++	++	+	++
+	+	+	+	+
++	++	++	++	++
+++	+++	++	++	++
+	+	+	+	+
+	+	+	+	+
++	++	++	++	++
+	+	−	+	+
+	+	−	+	+

Rhodymenia palmata (Apr. 24)	*Lomentaria catenata* (Feb. 1)	*Delesseria violacea* (May 2)	*Polysiphonia senticulosa* (May 2)	*Chondria crassicaulis* (Feb. 1)
+	+	+	+	+
+	++	++	++	+
+	++	++	++	+
++	++	++	++	++
++	++	++	++	++
++	++	++	++	++
+	+	+	−	−
+++	+++	+++	++	++
+	+	+	+	+
++	++	++	++	++
++	++	++	+	+++
+	+	+	++	+
+	+	+	++	+
	++	++	+	++
+	+	+	−	+
+	+	+	+	+

hydrochloric acid. The alcoholic extracts and protein hydrolysates were concentrated and evaporated to dryness. The amino acids present in these preparations were investigated qualitatively using two-dimensional paper chromatography. Aliquots containing 0.5 mg of amino acids were used for each chromatogram. Tōyō No. 2 filter paper sheets (40 cm×40 cm) were used throughout the present experiments. One hundred and two gm of phenol which were added 15ml of 0.1 per cent ammonium hydroxide solution were used as the first developing solvent, and the n-butanol-acetic acid-water (4:1:2) as the second solvent.

RESULTS AND DISCUSSION

The accompanying tables show the relative intensities of the amino acid spots obtained after ninhydrin treatment of the developed chromatograms.

According to the results shown in Table 1, the differences were fairly observable respectively between each of the marine algae, namely: *Undaria pinnatifida* f. *distans*, *U. pinnatifida* f. *narutensis*, *Hijikia fusiformis*, *Sargassum sagamianum* and *S. confusum* have comparatively few free amino acids; *Enteromorpha Linza* var. *cripata*, *E. intestinalis*, *Leathesia difformis*, *Desmarestia ligulata*, *Chorda Filum*, *Laminaria japonica*, *Sargassum Thunbergii*, *Iridophycus laminarioides*, *Delesseria violacea* and *Polysiphonia senticulosa* have more free amino acids; *Ulva pertusa*, *Enteromorpha Linza*, *Chaetomorpha moniligera*, *Scytosiphon Lomentaria*, *Porphyra Okamurai*, *P. crispata*, *P. tenera*, *P. pseudolinearis*, *P. umbilicalis*, *Neodilsea Yendoana*, *Grateloupia filicina*, *G. livida*, *Cirrulicarpus Gmelini*, *Gracilaria verrucosa*, *Rhodymenia palmata*, *Lomentaria catenata* and *Chondria crassicaulis* have the most numerous free amino acids among these marine algae.

Chaetomorpha moniligera, *Chorda Filum*, *Laminaria japonica*, *Porphyra Okamurai*, *P. tenera*, *P. umbilicalis*, *Grateloupia livida*, *Rhodymenia palmata*, *Lomentaria catenata*, *Delesseria violacea* and *Polysiphonia senticulosa* differ in their contents of free glutamic acid from those of other marine algae. *Chaetomorpha moniligera*, *Scytosiphon Lomentaria* and *Porphyra tenera* show dominant presence of free glycine and threonine, while *Laminaria japonica*, *Porphyra Okamurai*, *P. tenera* and *Grateloupia livida* are rich in free alanine, and lastly *Chorda Filum*, *Porphyra tenera* and *Rhodymenia palmata* are abundant in free aspartic acid contents.

As to the free amino acid occurring in marine algae, in general, aspartic acid, glutamic acid, serine, glycine, threonine, alanine and proline are comparatively rich. Then cystine, tyrosine, valine, methionine, phenylalanine, leucine, tryptophan, arginine, lysine and histidine are absent or present in only small amounts in their free state if they do occur.

On the other hand, Table 2 shows that cystine, aspartic acid, glutamic acid, serine, glycine, threonine, alanine, valine, methionine, phenylalanine, leucine, proline and arginine

are commonly contained as combined amino acids in marine algae. Tyrosine, lysine and histidine appear to be absent in combined state in some species of marine algae. However, even when ninhydrin colour reaction is negative, there is insufficient basis for a conclusion that these amino acids never occur in marine algae, because the minimum detectable amounts of these amino acids with ninhydrin are great. Tryptophan was detected on chromatograms of 5 N barium hydroxide hydrolysates of the marine algae. The author confirmed its presence by using P-dimethylaminobenzaldehyde in strong hydrochloric acid as a spraying reagent, whereupon it appeared as a blue-coloured spot.

Large amounts of aspartic acid, glutamic acid, serine, glycine, alanine, valine, phenylalanine, leucine and arginine proved to be present as constituents of algal protein. Especially, valine, phenylalanine and leucine occur without exception in large amounts as combined amino acids in all the marine algae examined, notwithstanding the fact that these amino acids are absent or occur only in small quantity as free amino acids. Lastly the contents of cystine, methionine, proline, tryptophan, lysine and histidine appeared to be less in quantity as compared with those of the amino acids above mentioned.

SUMMARY

The present paper deals with the results of experiments on free and combined amino acids occurring in five species of marine green algae, 11 species of marine brown algae and 16 species of marine red algae. Differences in amounts and varieties of various amino acids were observed as shown in Tables 1 and 2.

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