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CHEMICAL STUDIES ON MARINE ALGAE

XII. The Free Amino Acids in Several Species of Marine Algae

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Abstract

It may be reasonable to remark that the free amino acids in marine algae represent the greater part of non-protein nitrogenous material, though in it nitrate nitrogen and organic bases are both found to be contained in small amount. The forms of nitrogen contained in marine algae are characterized to be comparatively rich in non-protein nitrogenous material. The tasty ingredients in "Asakusanori", *Porphyra tenera* enjoyed with great relish and in *Laminaria japonica* are attributed to free amino acids occurring in that material.

Recently Y. Tsuchiya and Y. Suzuki¹⁾ have proven by means of bioassay that the main constituents of the tasty ingredients in "Asakusanori" are glutamic acid and alanine existing freely. C. Ogino²⁾ using paper chromatography has revealed that glutamic acid, aspartic acid, alanine, cystine and arginine are widely contained in free form in many species of marine algae.

C. B. Coulson³⁾ has also investigated the free amino acids in nine species of brown and red algae, and reported that glutamic acid, aspartic acid, glycine, alanine, valine, leucine and proline widely occurred; that there was no cystine, serine, threonine, phenylalanine, arginine and lysine present in some species of those algae nor did either tyrosine or histidine occur in any species.

In this study the free amino acids in *Ulva pertusa*, *Laminaria japonica*, *Iridaea cornucopiae* and *Porphyra tenera*, assuming tentatively as the representatives of the green, brown and red algae have been determined quantitatively by the technique of ion exchange of Moore and Stein.⁴⁾

Experimental

Methods—Materials were collected at Nanaehama, in the vicinity of Hakodate with the exception of dried materials of *Porphyra* obtained from the open market. After having been collected, the fresh marine algae were washed repeatedly with distilled water, dried at room temperature and at 100°C for thirty minutes in a draft, and then powdered. Free amino acids were extracted by shaking the dried, ground weed with 75 per cent of ethanol for 24 hours, in the proportion of 1 gm. to 100 ml. After this treatment had been repeated three times, the extracts were mixed, concentrated *in vacuo* and filled up to 10 ml.

Total nitrogen and ethanol extract nitrogen were estimated by the micro-Kjeldahl

procedure, amino nitrogen by the VanSlyke procedure.

Free amino acids were determined quantitatively by the technique of ion exchange of Moore and Stein. They were also identified by the ascending technique of one-dimensional paper chromatography.

N-butanol-acetic acid-water (4:2:1) was employed as the solvent.

Results

The time of collection and the forms of nitrogen determined are shown in Table 1.

Table 1. Forms of nitrogen in various algae

Species	Class	Time of collection	Total N in dried material	Ethanolic extract N in dried material	Free amino N in dried material	Ethanolic extract N	Amino N
						Total N	Ethanolic extract N
			<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
<i>U. pertusa</i>	Green	May 8	5.305	0.437	0.206	8.2	47.1
<i>L. japonica</i>	Brown	Apr. 25	1.593	0.185	0.132	11.6	71.3
<i>I. cornucopiae</i>	Red	Apr. 24	3.009	0.589	0.168	19.5	28.5
<i>P. tenera</i>	Red	—	6.664	1.005	0.574	15.1	57.1

According to the results as shown in Table 1, the ethanolic extract nitrogen in *Porphyra* and *Iridaea* accounted for 15 and 20 per cent of the total respectively, being considerably abundant as compared with its value in *Laminaria*.

In *Ulva*, though the total nitrogen was very great, the ethanolic extract nitrogen accounted for 8 per cent of the total, showing the minimum value among these algae determined. A similar fact has been recognized in *Ulva lactuca* by D. G. Smith and E. G. Young⁵. The amino nitrogen in *Porphyra* and *Laminaria* was also found to represent 57 and 71 per cent, respectively, of the ethanolic extract nitrogen. The amino nitrogen in *Iridaea* is much less than that of the above two marine algae, viz. its ratio to the ethanolic extract nitrogen showed only 28 per cent.

In Fig. 1 are illustrated the sections of effluent curves obtained with an aliquot of the ethanolic extract of *Ulva* equivalent to about 50 mg. of amino acid on a column of Dowex 50 resin. The distribution of the nitrogen of the free amino acids in the four species examined is reported in Table 2 as mg. percentage of the dried materials. Table 3 gives nitrogen of the amino acids as percentages of total amino nitrogen in ethanolic extract of these marine algae.

Discussion

According to the results obtained as shown in Table 3, the distribution of the free amino acids in alcoholic extract of four species of marine algae is widely divergent. Namely, the proportion of glutamic acid and alanine in *Iridaea* and *Laminaria* is

Table 2. Free amino acids in various algae
The amino acid are expressed as mg percentages of the dried material.

Amino acid	<i>Ulva pertusa</i>	<i>Laminaria japonica</i>	<i>Iridaea cornucopiae</i>	<i>Porphyra tenera</i>
Alanine	85.1	155.7	106.1	942.7
Arginine	181.7	0.0	0.0	56.5
Aspartic acid	133.4	45.8	59.9	817.2
Asparagine	0.0	56.0	51.6	214.4
Citrulline	0.0	0.0	0.0	240.9
Cysteic acid	12.7	7.1	32.9	34.5
Glycine	22.1	8.8	9.5	89.1
Glutamic acid	114.0	353.9	554.9	768.2
Phenylalanine	30.3	0.0	81.2	0.0
Proline	107.1	104.4	0.0	111.8
Serine	102.5	30.7	18.4	40.4
Threonine	65.7	60.9	93.4	154.7
Tryptophan	0.0	0.0	0.0	132.5
Tyrosine	31.2	0.0	13.6	0.0
Valine	0.0	0.0	0.0	0.0
Ammonia	75.6	43.1	17.8	48.3
Total	961.4	866.4	1039.3	3651.2

Table 3. Free amino acids in various algae
The amount of nitrogen of the amino acids is expressed as percentages
of total amino nitrogen in alcoholic extract.

Amino acid	<i>Ulva pertusa</i>	<i>Laminaria japonica</i>	<i>Iridaea cornucopiae</i>	<i>Porphyra tenera</i>
Alanine	6.5	18.5	9.9	25.8
Arginine	28.3	0.0	0.0	3.2
Aspartic acid	6.8	3.7	3.7	15.0
Asparagine	0.0	9.0	6.5	7.9
Citrulline	0.0	0.0	0.0	10.1
Cysteic acid	0.5	0.5	1.6	0.5
Glycine	2.0	1.2	1.1	2.9
Glutamic acid	5.3	25.5	31.4	12.7
Phenylalanine	1.2	0.0	4.1	0.0
Proline	6.3	9.6	0.0	2.4
Serine	6.6	3.1	1.5	0.9
Threonine	3.7	5.4	6.5	3.2
Tryptophan	0.0	0.0	0.0	1.6
Tyrosine	1.2	0.0	0.6	0.0
Valine	0.0	0.0	0.0	0.0
Ammonia	30.1	26.9	8.7	6.9
Total	98.5	103.4	75.6	93.1

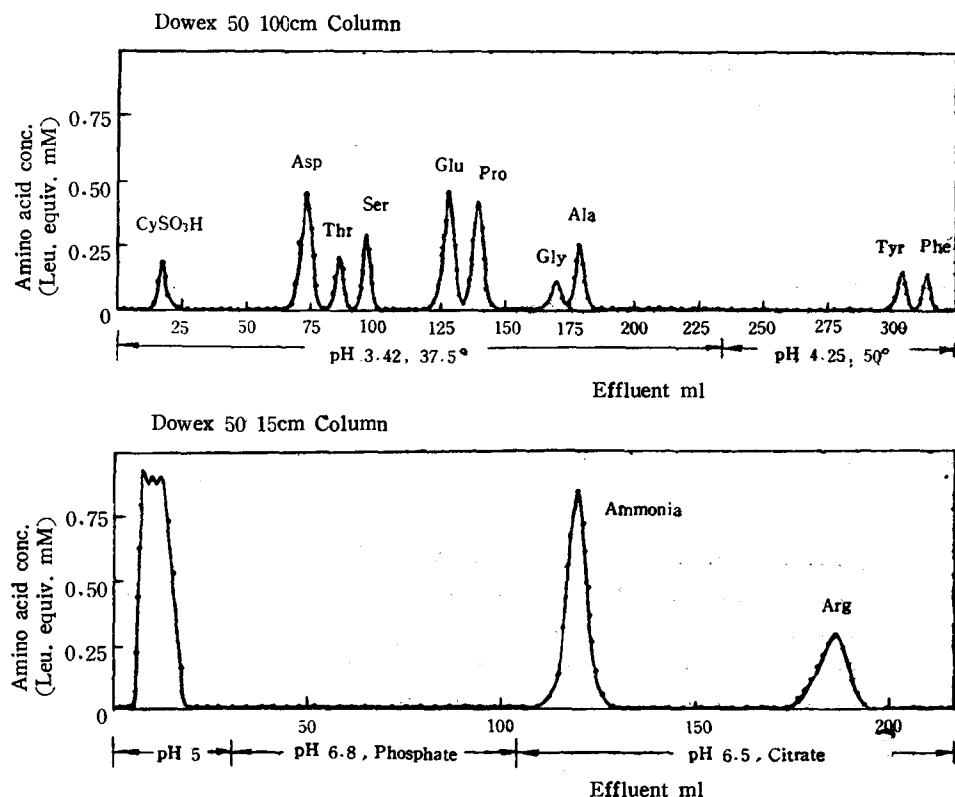


Fig. 1. Section of effluent curves obtained with alcoholic extract of *Ulva Pertusa* on a column of Dowex 50 resin.

remarkably higher than in *Ulva*, showing respectively 41 and 44 per cent of the total amino nitrogen in their alcoholic extracts. This tendency is also recognized in *Porphyra*, though reverse relation between the content of glutamic acid and alanine is observed.

Porphyra and *Ulva* differ from *Laminaria* and *Iridaea*, in the content of aspartic acid which predominates over that of glutamic acid. *Ulva* shows remarkable high content in arginine and serine, but none of asparagine in alcoholic extract. The presence of citrulline has been recognized specifically in alcoholic extract of *Porphyra*. This amino acid had been recognized heretofore in alcoholic extract of *Enteromorpha Linza* by C. Ogino,²⁾ and in acid hydrolysates of the insoluble material of *Chondrus crispus* by D. G. Smith and E. G. Young.⁵⁾ The proline content is high in *Ulva* and *Laminaria*, but this amino acid in *Iridaea* and *Porphyra* appears to be either completely absent or it occurs in only small amounts. The alcoholic extract of marine algae is also remarkable for its appreciable content of the sulfur-containing amino acid cysteic acid.

In the estimation of the free amino acid in *Ulva* and *Laminaria*, ammonia content was found to be markedly higher than that of the other species. This fact seems to be

undesirable for representing the distribution of the free amino acids in alcoholic extract of these two marine algae. It is thus presumed that the content of ammonia should be less than 10 per cent of total amino nitrogen in alcoholic extract, provided the dried materials of these marine algae were prepared rapidly with much more than the usual care.

Summary

The distribution of the free amino acids in 75 per cent alcoholic extract of four species of marine algae: *Ulva*, *Laminaria*, *Iridaea*, and *Porphyra*, has been determined by quantitative chromatography. The composition of the free amino acids in these marine algae was found to differ markedly according to species.

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