THE FREE AND COMBINED AMINO ACID CONTENTS IN SPECIES OF *CAULERPA* FROM SOUTHEAST COAST OF INDIA

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[*Caulerpa racemosa* var. *macrophysa*, *C. racemosa* var *cornyphora* and *C. scalpelliformis* are analysed by quantitative paper chromatographic technique for their amino acid contents in proteins, peptides and free state. It is found that no appreciable variation occurs in the quality of amino acid make up in these algae; but quantitative differences are apparent in them. Moreover, both qualitative and quantitative variations occur in the amino acid make up of the peptides, and in the free state. The results are compared with those of the other investigations.]

INTRODUCTION

In continuation of previous work on the genus: *Caulerpa* (Lewis and Gonzalves, 1959 a, b, 1960) the amino acid constituents in some additional specimens of this genus are investigated in order to throw more light on the proteins, peptides and free amino acid contents. The algae investigated here are. *Caulerpa racemosa* (Forsk) W. v. B. var. *macrophysa* Kuetz., *C racemosa* (Forsk.) W. v. B. var. *cornyphora* (Mont.) W. v. B. and *C. scalpelliformis* (Rr. Br.) W. v. B. The specimens were collected from southeastern coast of India, in the months of February and March.

MATERIALS AND METHODS

The plants were collected, preserved and analysed as described earlier (Lewis and Gonzalves, 1962)

RESULTS

The results are given in Tables I, II and III. The amino acids which were not quantitatively determined because of diffusion of the bands or interference of other compounds or the minuteness of their quantities are denoted by the "+", "++" etc. signs. An increasing number of these signs indicate relatively larger concentration of these with the color reagents used; the depth of color being judged merely by visual colour comparison. Although tryptoph an and hydroxyproline occured in estimable amounts especially in the protein hydrolysates, due to inadequacy of available equipments, these were not estimated *in toto*, and are merely indicaed by the "+" signs. Moreover, leucine and isoleucine were estimated together, and are referred to as "leucine (s)".

DISCUSSION

All the twenty-three amino acids detected in the protein hybrolysates occured consistently in the specimens studied except β- alanine and homocystine (cf. Lewis and Gonzalves, 1662, p. 309, for the comments of the occurrence of y - amino-butyric acid, β- alanine, homocystine and ornithine in the protein hydrolysates of marine algae) Among the compounds estimated, α-alanine,

aspartic acid, gluatmic acid, leucine (s), proline, serine and valine did not vary appreciably in amount in the proteins of these plants although the others varied insignificantly. Despite these differences in the constituents, usually aspartic acid, glutamic acid, histidine, leucine (s), phenylalanine and proline occured in large amonnts in these algal proteins, while the others were in fairly large quantities except β-alanine, y-aminobutyric acid and homocystine which were usually in low concentrations.

Both qualitative and quantitative variations were observed in the amino acid constituents in the peptides of these algae. Of the eighteen compounds detected only eight occured in all the three specimens, while only leucine (s), proline and valine were found in estimable amounts. Among these specimens, although, *C. racemosa* var. *macrophysa* was least proteinaceous one, was fouud to be most concentratad in the peptides. Though all the commonly occurring amino acids constituents in the proteins were recroded in the peptide hydrolysates of these algae either consistently or occasionally, glycine, hydroxyprolnie ornithine and tryptophan (since all the samples might have been destroyed, cf. Lewis and Gouzalves, 1962) were not detected in any of the samples. However cysteic acid which is not a protein constituent of the algae, was found in estimable amount in *C. racemosa* var. *macrophysa*.

Although the amount of free amino acids remained the same in all the three specimens studied, variations were observed in amouut of individual compounds. Of the sixteen detected constituents (including carnosine, a dipeptide) only nine were consistently found, while only α - alanine, glutamic acid, lysine and proline occurred in estimable amounts in all the three algae. Though a majority of the amino acids found in the protein hydrolysates of these specimens was detected either consistently or occassionally in the free state, β - alanine, y - aminobutyric acid, arginine, glycine, hydroxyproline, homocystine, ornithine, phenylalanine and threonine escaped detection in them. In addition, carnosine occurred as free compound only in *C. racemosa* var. *cornyphora*.

A brief comparison of these results with those of the other workers on the genus: *Caulerpa*, is made below:

Mazur and Clarke (1942) studied the amino acid composition in *C. racemosa* after formic acid hydrolysis, and estimated arginine, cystine, methionine, tryptophan and tyrosine. Lewiz and Gonazalves (1959a) analysed separately the erect and creeping fronds of three species of *Caulerpa*: *C. racemosa* var. *uvifera f. condensata*, *C. peltata* var. *typica* and *C. sertularioides* f. *typica* for protein hydrolysates, and estimated consistently all the amino acids recorded by Mazur and Clarke (*loc. cit*,) except cystine (determined quantitatively in the samples of C. *sertularioides* f. *typica*) and tryptophan which were only detected in them. In addition, alanine, aspartic acid, glutamic acid, glycine, isoleucine, leucine, lysine, phenlyalanine, proline, serine, threonine and valine were estimated in all the samples, while hydroxyproline (except in the erect fronds of *C. racemosa* var. *uvifera* f. *condensata*) and ornithine were detected consistently. Later, Lewis and Gonzalves (1960) investigated the protein hydrolysates of the entire plants of the above species and reported similar results as regards the qualitative composition of amino acids in them.

All the protein constituents recorded by the earlier workers (*loc. cit*) in the species of *Caulerpa* are estimated in the protein hydrolysates of all the specimens studied here except tryptophan and hydroxyproline which are detected only. In addition

y- aminobutyric acid is quantitatlvely determined in all the specimens: β- alanine, estimated in C. *scalpelliformis* and and detected in C. *racemosa* var, ***cornyphora***, while homocystine, estimated in the last mentioned variety only.

In the entire piants as well as erect fronds of C. *racemosa* var. *uvifera* f. *condenscta*, *C. peltata* var. ***typica*** and C. ***sertularioides*** f. ***typica***, Lewis and Gonzalves(1959b) have recorded nineteen free amino acids, of which, thirteen were consistently found in the entire plants.

In the algae studied here, although all the free amino acids recorded by Lewis and Gonzalves (1959b) occurred either cosistenly or occasionally in the free state, arginine, glycine, hydroxyproline, ornithine and phenylalanine escaped detection in all the specimens. In addition, tryptophan is detected here in all the samples, while carnosine in C. ***racemosa*** var, ***cornyphora*** only.

Lewis and Gonzalves (1959 a, b, 1960) observed that the amino acid constituents of the protein hydrolysates in the species of ***Caulerpa***, as well as in the differrnt regions of the same plant vary little in quality; but significiant differences were noticed in their amounts. The free amino acids varied both in quality and quantity in the different species as well as different regions of the same species. However, they felt the variations observed might have been due to the escape of sap which occurred while cutting the algae prior to drying the specimens.

Thus, it can be said, in the different species and varieties (as well as in the different regions) of *Caulerpa*, little variation occurs in the amino acid composition of protein hydralysates, as regards quality; but the majority of the constituents vary in amount, while amino acid constituents in the peptides and free state, vary both in quality and quantity. However, some additional amino acids recorded in the protein hydrolysates might have been due to the improved techniques made use in this investigation (cf. Lewis and Gonzalves, 1962) rather than their rarity in the specimens.

The specimens investigated here are comparatively poorer in the amount of proteins and free amino acids than those reported by Lewis and Gonzalves (1959a, b, 1960). This might have been due either to the ecological variations of the specimens studied or to the differences in the stage of the algal growth. In order to throw more light on this aspect, C. *sertularioides* f. *typica* collected at fortnightly intervals from Bombay, is being studied, and the results would be published elsewhere.

Summary

The varieties of *Caulerpa racemosa* and *C. scapelliformis* collected from south eastern coast of India, are studied for their proteins peptides and free amino acid contents using quantitative paper chromatographic technique.

Amino acid constituents in the proteins varied little among these specimens as regards quality, while peptide hydrolysates, and free amino acids showed significant variations both in quality and quantity.

These results are compared with those of the other workers in the field.

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TABLE — I. COMPARATIVE ACCOUNT OF THE PROTEIN HYDROLYSATES IN SPECIES OF *CAULERPA* FROM SOUTHEASTERN COAST OF INDIA

(Calculated as amount in grams per 16 g of portein N)

Compound	Caulerpa racemosa var. macrophysa	Caulerpa racemosa var. cornyphora	Caulerpa scalpelliformis
∝—Alanine	4.35	4.08	4.11
β—Alanine		+	0.23
y—Aminobutyric acid	0.76	0.47	0.67
Arginine	3.03	2.45	5.95
Aspartic acid	9.36	8.11	8.42
Cystine	3.12	3.56	1.80
Glutamic acid	8.79	8.51	8.85
Glycine	6.34	4.51	5.36
Histidine	4.82	8.23	7.21
Homocystine		1.46	
Hydroxyproline	+++	++	+++
Leucine (s)	6.90	6.14	6.54
Lysine	2.27	1.66	2.35
Methionine	1.32	3.36	2.58
Ornithine	0.66	2.73	0.35
Phenylalanine	5.67	6.93	7.09
Proline	7.47	6.53	7.52
Serine	4.73	4.43	4.15
Threonine	4.44	4.70	3.88
Tryptophan	++	++	+ +
Tyrosine	2.93	4.24	4.63
Valine	3.22	3.17	3.64
Number detected	21	23	22
Number estimated	19	20	20
Amount in grams per 16 g of protein N	80.18	85.32	85.33
Amount in grams per 100 g of the dry alga	8.48	21.56	21.78
Protein N in grams per 100 g of the dry alga	1.69	4.04	4.09
% Recovery of protein N	68.91	73.78	88.13

TABLE — II. COMPARATIVE ACCOUNT OF THE PEPTIDE HYDROLYSATES OF *CAULERPA* FROM SOUTHERN COAST OF INDIA

(Calculated as micrograms per gram dry weight of the alga)

Compound	Caulerpa racemosa var. macrophysa	Caulerpa racemosa var. cornyphora	Caulerpa scalpelliformis
∝-Alanine	3.55	0.72	++
y- Aminobutric acid	1.13	1.02	++
Arginine	8.31	+÷	++
Aspartic acid	3.41		
Cystine	+		
Cysteic acid	2.71		
Glutamic acid	8.74		
Histiidina	++	++	1.40
Leucind (s)	8.67	1.03	++
Lysine	++	++	
Methionine	7.03		
Phenylalanine	12.20		1.42
Proline	13.49	11.70	
Serine	6.10		
Threonine	4.87		
Tyrosiue	3.51	++	+
Valine	2.98	0.62	1.39
Number detected	18	10	9
Number estimated	15	6	4
Amount estimated	75.72	15.09	4.21

TABLE -- III. COMPARATIVE ACCOUNT OF THE FREE AMINO ACIDS IN SPECIES OF *CAULERPA* FROM SOUTHEASTERN COAST OF INDIA

(Calculated as micrograms per gram dry weight of the alga)

Compound	Caulerpa racemosa var. macrophysa	Caulerpa raeemosa var. cornyphora	Caulerpa scalpelliformis
∝- Alanine	1.03	0. 40	0.94
Aspartic acid	3.95		++
Carnosine		++	
Cystine	++	+	++
Glutamic acid	3.46	3.56	5.28
Histidine		+	
Leucine (s)	0.35	++	
Lysine	2.36	1.54	2.00
Methienine		2.50	
Proline	5.19	6.46	0.49
Serine	1.10	++	
Tryptophan	++	+++	+
Tyrosine	++	0.76	
Valine	0.50	0.62	+
Number detected	12	14	9
Number estimzted	9	8	4
Amount estimated	17.64	15.84	17.71