The Protein, Peptide and Free Amino-Acid Contents of Some Species of *Gracilaria* from South-East Coast of India

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[Four species of *Gracilaria* are investigated for their free amino-acid contents, as well as amino-acid constituents in the proteins and the peptides, using quantitative paper chromatographic technique.

Amino-acid constituents of different species of *Gracilaria* differ only in amount, while free amino-acids and the amino-acids in the peptides vary both in quality and quantity. A number of amino-acids recorded as protein constituents have even escaped detection in the peptides, while in the free state they occur either in all the species or in some only except homocystine. Moreover, some amino-acids occur exclusively in the free state.]

Introduction

In continuation of previous work (Lewis and Gonzalves, 1962), the free amino-acids, and the amino-acid contents of the proteins and peptides in the species of *Gracilaria* have been studied to ascertain whether any qualitative and quantitative variation occur in them. The species investigated are : *G. compressa* (Ag.) J. Ag., *G. confervoides* (Linn.) Grev., *G. lichenoides* (Linn.) Harv. and *G. corticata* J. Ag.

Experimental Procedure

The specimens were collected from southeast coast of India, in the months of February and March. These were cleaned, dried, powdered, preserved and analysed as has been described earlier (Lewis and Gonzalves, 1962).

Results

The results are given in Tables I, II, III and IV. The data are presented on similar basis as has been enumerated in the previous paper (Lewis and Gonzalves, 1962).

Discussion

Of the twenty-three amino-acids detected as protein constituents (cf. Lewis and Gonzalves, 1962. p. 309, for comments on the occurrence of β-alanine, y-aminobutyric acid, homocystine and ornithine) in the species of *Gracilaria*, all but β-alanine occurred con-

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sistently. These compounds were consistently estimated except hydroxyproline and tryptophan which were consistently detected (Lewis and Gonzalves, 1962). However, β-alanine occurred in estimable amount in *G. confervoides* and *G. lichenoides*. In the algae studied, the estimated amount of amino-acid constituents of proteins, and protein N (both based on per cent dry weight of the alga) ranged respectively between 4.91 or 0.85 (*G. compressa*) and 17.44 or 3.38 (*G. corticata*) ; amino-acids per 16 g. of protein N, and per cent recovery of protein N was respectively between 82.49 or 87.89 (*G. corticata*) and 95.16 or 94.90 (*G. lichenoides*).

The protein hydrolysates did not vary in quality among the different species of *Gracilaria* ; but they varied in quantity. Glycine, proline, serine and threonine were most concentrated in *G. compressa* than in the others ; arginine, leucine(s) and tyrosine, in *G. confervoides* ; α-alanine, β-alanine aspartic acid, glutamic acid, histidine, homocystine, methionine, phenylalanine and valine, in *G. lichenoides*, while y-aminobutyric acid, cystine lysine and ornithine, in *G. corticata*. Despite these variations, usually among the estimated compounds arginine, histidine, glutamic acid, aspartic acid, leucine(s) occurred in large quantities in all the four species ; α-alanine, cystine, methionine, phenylalanine and proline were in similarly large amounts in few species only, while the other constituents were usually in considerable amounts in them except β-alanine, y-aminobutyric acid and ornithine which were in tracer quantities.

Pillai (1957) studied the acid hydrolysates of *Gracilaria lichenoides* and estimated alanine, arginine, aspartic acid, glutamic acid, leucine(s), phenylalanine, serine, threonine tyrosine and valine, in both the young and mature specimens, while histidine was estimated in mature specimens only. Lewis and Gonzalves (1960), studied the protein hydrolysates of *Gracilaria foliifera* and estimated all the amino-acids recorded by Pillai (*loc. cit.*) except glutamic acid ; but in addition, glycine, lysine, methionine and ornithine were estimated, while cystine and tryptophan were detected. In the present investigation all the amino-acids recorded by the above cited workers (Pillai, 1957 ; Lewis and Gonzalves, 1960) are consistently estimated except trptophan, which was consistently detected. In addition, y-aminobutyric acid and homocystine are estimated in all the four species, while hydroxyproline is detected consistently.

In all, fifteen amino-acids were detected as peptide constituents. Of these, nine occurred consistently, while of the twelve compounds estimated only seven were estimated in all the four species. The total amount determined in them ranged between 0.0023% (*G. lichenoides*) and 0.01% (*G. corticata*).

The peptide hydrolysates varied both in quality and in quantity among the different species of *Gracilaria*. All the amino-acid constituents of the proteins occurred consistently or occasionally in the peptides except β-alanine, glycine, homocystine, hydroxyproline, ornithine, threonine and tryptophan (since all the peptide samples were analysed after acid hydrolysis, tryptophan in them might have been destroyed). In these algae usually proline alone had contributed nearlyhalf the peptide amount.

Of the twenty-four free amino-acids detected, eleven were found in estimable amounts, while only α-alanine, serine and valine occurred in estimable amounts in all

the four species. The total amount determined in them ranged between 0.04% (*G. lichenoides*), and 0.085% (*G. corticata*).

The free amino-acids varied both in quality and quantity in these algae. None of the constituents was consistently in large amount ; but comparatively serine was in large quantity except in *G. lichenoides*. Although arginine was seldom recorded in large amount in the free state in any seaweeds (Lewis and Gonzalves, 1959a, b, 1962a, b ; Lewis, 1962) it occurred in large quantities in *G. corticata*, and in which it contributed about three quarters of the total free amino-acids.

All the amino-acids recorded in the protein hydrolysates occurred either consistently or occasionally in the free state except homocystine. Moreover, 2-aminocaprylic acid and glycocyamine occurred exclusively in the free state in some of these species.

Table I shows a comparative account of the variations in the estimated amino-acids (when estimated at least in two different species) in proteins, peptides and free state in the different species of *Gracilaria*.

TABLE I. — Comparative Account of Variations in the Estimated Amino-Acids (when estimated at least in two different species) in species of *Gracilaria*

(Protein hydrolysates per 16 g. of protein N, while the other two are based on per cent. dry weight of the alga).

Compound	Proteins		Peptides		Free Amino-Acids	
	+	*	+	*	+	*
α–Alanine ...	4	2.08	4	22.72	4	14.05
β–Alanine ...	2	2.27				
n–Aminobutyric acid ...	4	1.86	4	8.96		
Arginine ...	4	1.40				
Aspartic acid ...	4	1.51			3	3.76
Cystine ...	4	1.85				
Glutamic acid ...	4	1.43	2	1.18	3	3.76
Glycine ...	4	2.05			2	3.40
Histidine ...	4	1.24	3	6.79		
Homocystine ...	4	2.42				
Leucine (s) ...	4	1.27	4	4.02	2	19.72
Lysine ...	4	1.68	3	7.08	3	17.77
Methionine ...	4	2.31	3	4.87	2	1.05
Phenylalanine ...	4	1.64				
Proline ...	4	1.83	4	3.83		
Serine ...	4	1 67			4	46.95
Threonine ...	4	1.83				
Tyrosine ...	4	1.62				
Valine ...	4	1.41	4	5.26	4	24.85
Total amount ...	4	1.15	4	4.34	4	21.36

† Indicates number of plants in which estimated

* Indicates ratio between the minimum and maximum amount (1 : X).

TABLE II — Comparative Account of Amino-Acid Constituents in Proteins of Different Species of *Gracilaria* from Southeast Coast of India

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

Compound	Gracilaria compressa	Gracilaria confervoides	Gracilaria lichenoides	Gracilaria corticata
α–Alanine ...	3.20	3.43	5.61	2.70
β–Alanine ...		0.56	1.27	
γ-Aminobutyric acid ...	0.57	0.51	0.64	0.95
Arginine ...	11.30	15.82	11.47	13.24
Aspartic acid ...	7.16	5.91	7.64	5.06
Cystine ...	4.90	5.18	3.82	7.05
Glutamic acid ...	7.72	6.59	7.77	5.44
Glycine ...	3.77	2.70	3.57	1.84
Histidine ...	8.85	8.61	10.57	8 51
Homocystine ...	1.32	1.52	3.19	2.93
Hydroxyproline ...	***	***	***	**
Leucine (s) ...	6.41	7.04	6 24	5.53
Lysine ...	2.26	1.86	2 42	3.12
Methionine ...	4.71	3.83	5 48	2.37
Ornithine ...	0 94	1.01	0.51	3.41
Phenylalanine ...	6.22	4.67	7.13	4.35
Proline ...	6.97	5 01	3.82	5.01
Serine ...	4.33	3.10	3.31	2.60
Threonine ...	4.33	2.65	3.44	2.37
Tryptophan ...	**	**	**	**
Tyrosine ...	3.58	4.90	3.06	3.03
Valine ...	3.96	3.66	4 20	2.98
Number detected ...	22	23	23	22
Number estimated ...	20	21	21	20
Amount in grams per 16 g. of protein N. ...	92.50	88.56	95.16	82.49
Amount in grams per 100 g. of dry alga ...	4.91	15.73	7.47	17.44
Protein N. in grams per 100 g. of dry alga ...	0.85	2.84	1.26	3.38
Per cent. recovery of protein N. ...	90.51	93.20	94.90	87.89

TABLE III — Comparative Account of Amino-Acid Constituents in Peptides of Different Species of *Gracilaria* from Southeast Coast of India
(Calculated as micrograms per gram dry weight of the alga)

Compound		Gracilaria compressa.	Gracilaria confervoides	Gracilaria lichenoides	Gracilaria corticata
α–Alanine	...	1.07	1.55	0.47	10.68
y–Aminobutyric acid	...	1.74	4.78	1.06	9.50
Arginine	...		*	**	16.36
Aspartic acid	...			0.87	*
Cystine	...			**	
Glutamic acid	...	0.62		0.73	
Histidine	...	**	7.37	2.75	18.66
Leucine (s)	...	3.75	4.93	1.69	6.80
Lysine	...	3.43	5.66	0.80	**
Methionine	...	2.21		0.78	3.80
Phenylalanine	...	*	**	**	
Proline	...	18.57	46.21	12.04	28.80
Tyrosine	...	**	**	0.85	**
Valine	...	1.37	2.82	1.04	5.47
Number detected	...	12	11	15	I2
Number estimated	...	9	8	12	9
Amount estimated	...	32.76	73.32	23.08	100.67

TABLE IV — Comparative Account of Free Amino-Acid Contents in Different Species of *Gracilaria* from Southeast Coast of India
(Calculated as micrograms per gram dry weight of the alga)

Compound		Gracilaria compressa	Gracilaria confervoides	Gracilaria lichenoides	Gracilaria corticata
α–A lanine	...	6.62	20.70	2.06	28.94
β–Alanine	...	**		*	
y–Aminobutyric acid	...			1.22	*
2–Aminocaprylic acid	...		**		
Arginine	...			*	605.02
Aspartic acid	...	7.66	12.92	**	28.80
Cystine	...	**	**	**	**
Glutamic acid	...	4.98	8 78		34.16
Glycine	...	8.32	28.26		
Glycocyamine	...				***
Histidine	...	**	3.81	**	
Hydroxyproline	...			**	
Leucine (s)	...	1.41	27.80	**	**
Lysine	...	3.65	8.21	**	64.86
Methionine	...	2.76		2.91	
Ornithine	...	2.94			
Phenylalanine	...	7.64	**	**	
Proline	...	***	***	***	***
Serine	...	30 29	168.08	3.58	75.02
Threonine	...			28.97	
Tryptophan	...	**	**	**	**
Tyrosine	...	6.04	**	**	**
Valine	...	1.80	19 88	0.80	8.58
Number detected	...	19	17	17	15
Number estimated	...	13	11	5	8
Amount estimated	...	84.11	329.41	39.54	845.38

Thus it can be said that although qualitative make-up of the protein constituents is similar to all the species of *Gracilaria*, these compounds not only vary in amount but also their magnitude of variation differ among these algae. The peptide hydrolysates and free amino-acids, both vary in quality and quantity. All but some amino-acid constituents of the proteins occur in peptides, while in the free state all are found either consistently or occasionally except homocystine. Moreover, some additional compounds also occur in the free state. As regards amount, proteins are most concentrated in all the species of Gracilaria, while peptides were in least quantity.

Although the variations are quite striking in the free amino-acids as well as in the amino-acids constituents of proteins and peptides, their exact taxonomical significance can be evaluated only after assessing the variations in these constituents in various species during different stages of their growth, as well as under diverse ecological conditions.

Since the protein contents of some of the species in this genus are considerable, while those of the others are less, for the nutritional evaluation of any algal genus it is better to study the protein contents of representative species of the genus rather than to rstrict the investigation to a few species only.

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