

PROTEIN, PEPTIDE AND FREE AMINO ACID COMPOSITION OF THE CALCAREOUS RED ALGAE *AMPHIROA ANCEPS* (LAMK.) DECSNE AND *AMPHIROA FRAGILISSIMA* (L) LAMOUR FROM PORT OKHA AND PORBANDAR

E. J. LEWIS

Central Salt and Marine Chemicals Research Institute, Bhavnagar, Gujarat, India

ABSTRACT

Amphiroa anceps (lamk.) Decsne and *A. fragilissima* (L) Lamour collected from port Okha and Porbandar are studied in detail for their free and combined amino acid composition. Though protein content and quantity of individual compound was similar in both algae, the former species is richer in phenylalanine, and latter in serine. *A. fragilissima* was richer of the two in total peptides. Moreover, almost all the compounds are more concentrated and only phenylalanine occur in large amounts in this alga. Non-fruiting *A. anceps* is rich in free amino acids. In this sample aspartic acid, glutamic acid and histidine occur in fairly large amounts while in the fruiting plants proline is predominant. Calcareous algal proteins seems to be distinctly poor in α -alanine, glutamic acid and proline compared to other algae.

INTRODUCTION

SEVERAL calcareous algae occur all along the Indian Coast (Krishnamurthy and Joshi, 1970), but so far there is no record of its detailed chemical composition (Rao, 1971). The present work is undertaken to study two common calcareous red algae viz., *Amphiroa anceps* (Lamk.) Decsne and *A. fragilissima* (L) Lamour for their free and combined amino acid composition, and to compare the results with the earlier reports as to the similarities and disparity with other marine algae with regards to these components. Furthermore, *A. anceps* was collected separately during immature and fruiting stages to ascertain the physiological changes in composition during these two stages in this alga.

The author wishes to acknowledge his thanks to Dr. D. J. Mehta, Director of this Institute for kindly permitting him to publish this work, while Dr. (Mrs.) Francisca Thivy, for confirming the identification of the specimen.

MATERIALS AND METHODS

Amphiroa anceps was collected separately in the months of October and March from Port Okha. The former specimens were young and nonfruiting while latter ones were mature and fruiting. *A. fragilissima* was collected from Porbandar in the month of December. These specimens were fruiting. The algal material was collected, cleaned, dried, powdered, preserved and analysed.

RESULTS

The results of the investigation are given in Tables 1, 2 and 3. In Table 1, amino acid composition in the protein hydrolyzates is given. The data represents amount of amino-N of each components in g/100 g of protein-N recovered. In Table 2, amino acid composition in the peptide hydrolyzates and Table 3, free amino

acid composition is given. The amount is expressed as $\mu\text{g/g}$ dry wt. of alga. The '-' indicates the compound is not detected, while '+' indicates the particular constituent is found below measurable level.

TABLE 1. Amino acid composition in the protein hydrolyzates in *Amphiroa anceps* (Lamk.) Decsne and *A. fragilissima* (L.) Lamour (of amino-N/100 g of protein-N recovered).

	<i>Amphiroa anceps</i>		<i>A. fragilissima</i>
	Immature plants	Fruiting plants	
α -Alanine	1.95	3.48	2.59
γ -Aminobutyric acid	1.26	—	0.20
Glycine	20.01	10.41	7.05
Isoleucine	1.66	2.90	2.41
Leucine	1.95	2.75	2.16
Valine	1.28	1.61	1.04
Serine	1.97	3.52	20.13
Threonine	1.72	4.41	2.47
Aspartic acid	9.42	15.00	14.09
Glutamic acid	3.06	4.56	3.34
Arginine	9.31	11.67	10.19
Citrulline	8.36	—	8.56
Lysine	4.00	2.97	2.30
Ornithine	1.95	2.25	1.56
Cysteic acid	3.58	0.89	0.41
Cystine	6.28	4.44	2.59
Methionine	1.17	1.10	0.37
Phenylalanine	3.38	5.83	0.89
Tyrosine	2.63	3.63	3.40
Histidine	13.78	16.54	12.52
Hydroxyproline	0.78	1.09	1.03
Proline	0.32	0.58	0.28
Tryptophan	0.18	0.35	0.43
Total number detected	23	21	23
Total amount estimated	100.00	99.98	100.01
Total protein recovered (% dry wt of alga)	6.25	6.71	4.45
Total protein-N recovered (% dry wt of alga)	0.98	0.94	0.65
Per cent recovery of protein-N	94.26	95.89	93.03

GENERAL OBSERVATIONS AND DISCUSSION

Proteins

As can be seen in Table 1, young and fruiting specimens contain similar amount of proteins, but γ aminobutyric acid and citrulline are absent in fruiting plants, while all the other twentyone amino acids are found to be common for both the samples. Young plants are significantly rich in glycine which alone accounts for a fifth of the total amount of protein hydrolyzates. The amount of this compound is reduced to an half this quantity in the fruiting plants. Quantities of individual amino acids remained similar in both the species studied except for serine and phenylalanine. The former of these two is more, while latter in lesser quantity in *A. fragilissima*. In spite of these variations histidine, aspartic acid, glycine and arginine occur in large amounts, and together accounts for nearly an half the total protein content.

Peptides

As can be seen in Table 2, *A. fragilissima* is comparatively rich in peptides. Although qualitative amino acid composition remained the same in both the species

practically all the compounds except lysine, cystine and methionine are found to be in greater concentration in *A. fragilissima*. It is interesting to note that citrulline occur in *A. anceps* during fruiting stage in the peptide while it is totally absent in proteins of the samples although proteins of nonfruiting specimens it occur comparatively large amounts. Individually all the amino acids occur in small amounts except for phenylalanine, which is found in considerable amounts in *A. fragilissima*.

TABLE 2. Amino acid composition in the peptide hydrolyzates in *Amphiroa anceps* (Lamk.) Decsne and *A. fragilissima* (L.) Lamour (Calculated as $\mu\text{g/g}$ dry wt of alga)

	<i>Amphiroa anceps</i>		<i>A. fragilissima</i>
	Immature plants	Fruiting plants	
α -Alanine	1.7	5.9	29.1
Glycine	3.5	11.4	50.7
Isoleucine	1.1	13.9	63.8
Leucine	1.4	9.6	42.9
Valine	1.3	6.6	50.7
Serine	2.6	5.6	17.9
Threonine	1.7	3.8	14.2
Aspartic acid	7.2	16.9	49.7
Glutamic acid	2.9	6.8	32.1
Arginine	1.6	3.2	27.0
Citrulline	—	2.7	—
Lysine	1.0	2.8	4.9
Ornithine	1.9	1.9	11.3
Cysteic acid	—	—	11.1
Cystine	1.8	26.1	1.8
Methionine	2.8	—	10.2
Phenylalanine	2.2	19.0	124.0
Tyrosine	—	6.6	35.2
Histidine	1.9	4.6	15.7
Proline	2.1	10.1	5.0
Total number detected	17	18	19
Total amount estimated	38.7	157.5	593.3
Total peptide-N estimated	5.47	20.23	76.05
Per cent of protein recovered	0.06 (0.06)	0.23 (0.22)	1.34 (1.17)

Figures in bracket are based on N
 — Not detectable

Free amino acids

As can be seen in Table 3, the non-fruiting plants contain practically twice the amount of the total free amino acids than that of fruiting *A. anceps*. γ -Aminobutyric acid, capryline and citrulline occur only in non-fruiting plants. Moreover, though most of the compounds are found in greater concentration in non-fruiting plants, the differences are much apparent in the amounts of glycine, dicarboxylic amino acids, arginine, lysine and histidine, while fruiting plants are strictly more concentrated in proline, nearly account for a three-fifths of the total amount of the amino acids, showing thereby concentration of free proline may have direct bearing on reproductive cycle of the plants. Except for slight higher concentration of leucine, isoleucine and valine in *A. fragilissima* none of the other amino acids show any variation in amount between two species.

Qualitatively amino acid composition in protein hydrolyzates compares well with earlier reported values (Lewis, 1967). Total protein content in these algae studied here though poorer than the red algae from Bombay (Lewis and Gonzalves, 1962 a, b) Japan (Takagi, 1953 a, b), and American Coast (Gotelli and Cleland, 1968), seaweeds, compares well with other reports on red algae (Pillai, 1957; Lewis, 1963 a, b, 1964; Nasr *et al.*, 1967). These algae are strikingly poorer in proline, glutamic acid and α alanine (Lewis, 1967), but strikingly rich in glycine.

TABLE 3. Free amino acid composition in *Amphiroa anceps* (Lamk.) Decsne and *A. fragilissima* (L.) Lamour ($\mu\text{g/g}$ dry wt of algae)

	<i>Amphiroa anceps</i>		<i>A. fragilissima</i>
	Immature plants	Fruiting plants	
α -Alanine	76.7	28.0	73.5
γ -Aminobutyric acid	39.3		6.8
Capryline	4.8		
Glycine	74.4	10.6	45.1
Isoleucine	8.0	7.7	40.2
Leucine	5.8	5.6	30.6
Valine	6.5	10.6	46.7
Serine	39.1	12.7	22.1
Threonine	31.8	8.3	47.4
Aspartic acid	205.5	42.2	209.4
Glutamic acid	333.5	62.7	100.1
Glutamine	—	5.4	9.6
Arginine	168.0	3.9	7.9
Citrulline	6.8		
Lysine	80.7	4.7	5.9
Ornithine	9.7	20.7	25.7
Cysteic acid	93.4	20.2	45.2
Cystine	37.2	20.8	31.1
Methionine	3.5	5.0	13.4
Phenylalanine	21.8	15.9	42.3
Tyrosine	40.5	6.6	29.7
Histidine	383.5	4.6	18.4
Hydroxyproline	22.4	54.3	
Proline	16.7	535.7	35.3
Tryptophan	2.1	3.1	2.1
Total number detected	24	22	22
Total amount estimated	1711.7	889.3	888.5
Total free amino-N estimated	287.6	109.2	108.1
Per cent of protein recovered	2.7 (2.93)	1.3 (1.77)	2.0 (1.66)

Figures in bracket are based on N.

— Not detectable

Although comparatively more number of amino acids occur in the peptides hydrolyzates but total amount compares well with the earlier reports (Lewis, 1967).

Free amino acid report agrees to certain extent earlier report (Lewis, 1967), but differs specially in the reported occurrence of glutamine over here. Moreover, differences noted during growth and reproductive phase confirm the earlier findings (Lewis, 1963; Lewis and Gonzalves, 1962 b).

REFERENCES

- GOTELLI, I. B. AND R. CLELAND 1968. Difference in the occurrence and distribution of hydroxyproline-proteins among algae. *Amer. J. Bot.*, **55**: 907-914.
- KRISHNAMURTHY, V. AND H. V. JOSHI 1970. A Check-list of Indian marine algae. *Bull. Central Salt & Marine Chemicals Research Institute, Bhavnagar*. pp. 1-37.
- LEWIS, E. J. 1963 a. Studies on the proteins, peptides and free amino acids in some species of red algae from southeastern coast of India. *Proc. natl. inst. Sci. India*, **29**: 137-145.
- 1963 b. Studies on the fortnightly analysis of the proteins, peptides and free amino acid contents in some marine algae from Bombay. *Ibid.*, **29**: 263-286.
- 1963 c. The proteins, peptides and free amino acid contents of some species of *Acanthophora* from southeastern coast of India. *Rev. algol.*, **7**: 237-241.
- 1964. The protein, peptide and free amino acid contents of some species of *Gracilaria* from southeastern coast of India. *J. Fish. Tech. India*, **1**: 158-163.
- 1967. A review on protein, peptide and free amino acid contents of Indian marine algae. *Proc. Seminar on Sea, Salt & Plants*. pp. 296-308.
- AND E. A. GONZALVES 1962 a. Periodic studies of the proteins, peptides and free amino acids in *Enteromorpha prolifera* f. *capillaris* and *Ulva lactuca* var. *rigida*. *Ann. Bot. N. S.*, **26**: 317-327.
- AND ————— 1962 b. The proteins, peptides and free amino acid contents of some species of marine algae from Bombay. *Ibid.*, **26**: 301-316.
- AND ————— 1962 c. Studies on the proteins, peptides and free amino acids in cystocarpic and tetrasporic plants of *Agardhiella robusta* from Bombay. *New Phytol.*, **61**: 288-290.
- NASR, A. H., I. A. BEKHEET AND R. K. IBRAHIM 1967. An electrochromatographic investigation of the amino acid pattern of some marine algae from Alexandria. **29**: 80-92.
- PILLAI, V. K. 1957. *Chemical studies on Indian seaweeds*, II. Partition of nitrogen. **45 B**: 43-63.
- RAO, M. U. 1971. The economic seaweeds of India. *Bull. Central mar. Fish. Res. Inst.*, **20**: 1-68
- TAKAGI, M. 1953 a. Chemical studies on marine algae. VII. Tyrosine and tryptophan contents in various species of marine algae. *Bull. Fac. Fisheries Hokkaido Univ.*, **4**: 86-91.
- 1953 b. Chemical studies on marine algae. VIII. Threonine and serine contents of various species of marine algae. *Ibid.*, **4**: 92-95.