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Amino acid composition of freshwater algae

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With 4 tables in the text

Abstract

The protein content of 13 species of freshwater algae ranged from 10.50 to 46.35 g/100 g dry wt. Proportions of individual amino acids in the protein of the different species were remarkably similar. Differences in protein content will influence the efficiency with which algal tissues are utilized by consumer and decomposer organisms.

Introduction

Most studies of plant production in freshwater ecosystems are concerned with the quantities of organic matter produced in a given length of time. Production estimates are expressed in terms of dry matter, organic matter, carbon or calories, but the efficiency of utilization of this material by consumer organisms is to a large extent dependent upon the nutritive quality. Therefore, information on the chemical composition and nutritive value of various species of plants is needed to supplement the data of primary productivity. Many chemical constitutuents are involved in the concept of food quality, but the amounts of protein and component amino acids are primary determinants of the food quality of plant materials (BOYD 1970; BOYD & GOODYEAR 1971; POLISINI & BOYD 1972). Since few data of the amino acid composition of freshwater vegetation are available, the present study was initiated to examine the protein and amino acid composition of freshwater algae from natural situations.

Materials and Methods

Samples of algae for amino acid determinations were collected from ponds on the Fisheries Research Unit, Agricultural Experiment Station, Auburn University. Samples of water which contained blooms of phytoplankton were centrifuged at 1500 rpm. The algal material in the centrifuge tubes was then freeze-dried, pulverized with a mortar and pestle and desiccated until used for analysis. All phytoplankton samples were collected from dense blooms in which one species comprised 99 % or more of the phytoplankton. Some zooplankton and organic detritus were present in all samples, but the amount of the dominant phytoplankter greatly exceeded the quantities of other substances. Samples of macrophytic algae were carefully removed from relatively unialgal communities. The samples were

Table 1. Amino acid and protein content of 13 species of freshwater algae. The values for *Anabaena circinalis* are the averages of four samples. All other values are for single samples of the particular species.

Amino acid	*Euglena proxima*	*Dictyosphaerium pulchellum*	*Chara vulgaris*	*Chara braunii*	*Chara fibrosa*	*Nitella* sp.	*Spirogyra* sp.	*Pithophora kewensis*	*Hydrodictyon reticulatum*	*Rhizoclonium hieroglyphicum*	*Anabaena circinalis*	*Microcystis aeruginosa*	*Aphanizomenon flos-aquae*	$\bar{x}$ + S. E.[a]	C. V.[b]
	g/100 g protein														
Lysine	8.03	6.54	6.92	6.84	7.76	6.82	5.78	7.75	7.93	7.10	6.81	5.23	7.13	6.97 ± 0.23	11.72
Histidine	1.98	1.92	1.80	2.54	2.15	2.57	2.05	2.14	2.13	2.15	1.87	1.40	2.11	2.06 ± 0.08	14.56
Arginine	4.75	6.47	5.88	5.80	7.24	5.11	4.15	4.81	7.18	5.18	6.58	9.71	7.44	6.18 ± 0.42	24.19
Aspartic acid	9.10	13.61	16.08	16.74	11.68	11.02	8.69	14.02	10.27	11.76	11.27	12.95	11.29	12.19 ± 0.64	19.11
Threonine	5.74	6.21	5.06	4.74	2.07	4.49	2.77	5.37	5.00	5.44	6.19	5.68	5.68	4.96 ± 0.34	25.00
Serine	4.32	6.01	5.69	5.04	2.79	4.59	4.49	5.65	4.90	5.85	4.95	5.21	4.94	4.96 ± 0.24	16.73
Glutamic acid	10.42	9.61	11.94	11.61	11.38	17.39	18.56	14.21	14.28	13.73	13.12	12.81	14.35	13.34 ± 0.71	19.12

Table 1 Continued

Amino acid															
	g/100 g dry wt.														
Proline	4.88	5.15	4.67	4.44	3.72	4.51	3.97	5.43	4.75	4.80	3.22	3.55	3.55	4.36 ± 0.19	15.83
Glycine	6.06	6.36	6.02	6.06	7.02	5.88	7.35	6.16	5.79	6.63	5.49	4.79	5.01	6.05 ± 0.19	11.74
Alanine	9.77	8.06	6.80	7.14	8.74	9.39	15.04	8.54	7.62	6.99	8.28	7.88	8.27	8.66 ± 0.59	24.50
Cystine	0.72	0.58	1.63	1.63	0.84	2.65	0.22	0.62	0.92	1.44	0.17	0.28	0.42	0.93 ± 0.20	77.42
Valine	6.00	6.14	6.00	5.48	6.77	4.85	4.71	6.21	5.46	5.82	6.18	6.20	5.81	5.82 ± 0.16	9.79
Methionine	2.56	0.86	1.29	1.83	2.07	1.57	1.50	1.74	2.28	2.22	1.76	1.73	2.03	1.80 ± 0.13	25.00
Isoleucine	4.87	4.25	3.67	3.84	5.00	3.55	4.12	4.38	3.91	4.30	5.87	5.57	5.69	4.54 ± 0.22	17.40
Leucine	9.11	9.24	7.73	8.05	9.85	7.21	7.90	8.48	8.42	8.07	9.39	8.75	9.11	8.56 ± 0.21	8.88
Tyrosine	6.09	3.62	3.86	3.39	5.01	3.55	3.41	0.66	3.74	3.77	4.16	4.00	3.87	3.78 ± 0.37	34.92
Phenylalanine	5.63	5.35	4.95	4.83	5.91	4.85	5.28	3.84	5.42	4.75	4.70	4.23	3.31	4.85 ± 0.20	15.05
	g/100 g protein														
Protein[c]	45.49	10.50	10.35	17.10	8.66	13.28	15.93	13.77	13.09	10.08	46.09	42.14	46.35	22.53 ± 4.56	70.17
Nitrogen	9.48	—	2.24	3.53	1.92	2.90	3.24	2.94	2.84	2.01	9.58	9.31	9.49	4.96 ± 0.97	67.94

[a] Average ± 1 standard error.
[b] Coefficient of variation.
[c] Sum of amino acids.

washed 5 to 10 times in pans of tap water and all debris removed. Microscopic examination revealed that the samples contained 99 % or more of one species. Small samples (5 to 10 g wet weight) were freeze-dried and prepared for analyses as indicated above.

Nitrogen analyses were performed by the micro-Kjeldahl technique (Association Official Agricultural Chemists, 1960). The procedure for amino acid analysis was essentially the same as that reported by GERLOFF, LIMA, & STAHMANN (1965). Small samples (30 to 50 mg) were weighed into glass tubes and 2 ml of 6 N HCl were added for each mg of crude protein (nitrogen × 6.25) in the samples. Tubes were sealed under vacuum and hydrolyzed for 20 hr at 110° C. Tubes were opened and the hydrolysates were dried at 40 C in a rotary evaporator. The residue was dissolved in a known amount of citrate buffer and an aliquot was analyzed for its amino acid content with a Beckman Spinco amino acid analyzer. Hydrolyses and acid analyses were performed by personel of the Department of Biochemistry, Vanderbilt University. Amino acid analyses were made of four samples of *Anabaena circinalis*. However, considerable time and expense were involved in the determinations and only one sample of each of the other species was analyzed for amino acid content.

Additional samples of three genera of macrophytic algae, *Spirogyra, Chara,* and *Pithophora*, were collected from ponds in Mississippi and Alabama. These samples were cleaned of debris as indicated above and oven dried at 60 C. Nitrogen analyses were made by the micro-Kjeldahl technique.

Results

Data for the amino acid and protein (sum of amino acids) composition of 13 species of algae are summarized in Table 1. Macrophytic chlorophyceans (*Chara, Nitella, Spirogyra, Pithophora, Rhizoclonium,* and *Hydrodictyon)* contained 10 to 20 g protein per 100 g dry weight. *Dictyosphaerium pulchellum*, a planktonic chlorophycean, contained 10.50 g protein/100 g dry wt., but the three planktonic cyanophyceans (*Anabaena, Microcystis,* and *Aphanizomenon*) and the phytoplankter *Euglena proxima* had 42 to 46 g protein/100 g dry wt. Protein values were 72.4 to 80.2 % of crude protein values (nitrogen × 6.25) for the respective samples. Tryptophan was destroyed during hydrolysis, but it is unlikely that the loss of tryptophan accounted of the over estimation of protein by crude protein. Nevertheless, there was a good correlation between crude protein or nitrogen and the sum of amino acids when all samples were considered ($r = 0.83$; $P > 0.01$).

The amounts of a particular amino acid in dried samples of different species varied considerably since there were large differences in the protein content of the samples. For example, *Euglena proxima* contained 3.65 g lysine/100 g dry wt, but *Chara vulgaris* contained only 0.72 g lysine/100 g dry wt. Therefore, for comparative purposes, values for each amino acid were converted to g amino acid per 100 g protein. In comparing the values for an amino acid across all species, the lowest value was usually ½ or less than the highest value but values for most species were of similar magni-

tude. Standard errors for the average of values for each amino acid in the different species were usually small and coefficients of variations were below 20 % for several amino acids. The greatest variation was encountered in the proportion of cystine in the protein of the different species. The protein of *Microcystis aeruginosa* contained a large amount of arginine and a particularly large quantity of alanine was present in the protein of *Spirogyra* sp. Comparatively small proportions of methionine and tyrosine were present in *Dictyosphaerium pulchellum* and *Pithophora kewensis*, respectively.

The protein content of samples of *Anabaena circinalis* from four different ponds ranged from 42.64 to 51.64 g/100 dry wt. (Table 2). Variation in the proportion of individual amino acids in the protein of the four

Table 2. Amino acid and protein content of four different samples of *Anabaena circinalis*.

Amino acid	1	2	3	4	$\bar{x} \pm$ S. E.[a]	C. V.[b]
			g/100 g protein			
Lysine	6.76	6.36	8.88	5.24	6.81 ± 0.76	22.32
Histidine	1.91	2.01	2.00	1.56	1.87 ± 0.11	11.23
Arginine	6.51	6.77	6.41	6.64	6.58 ± 0.08	2.43
Aspartic acid	10.98	10.64	10.64	12.83	11.27 ± 0.53	9.32
Threonine	6.36	5.81	5.89	6.69	6.19 ± 0.25	16.12
Serine	5.06	4.82	4.88	5.04	4.95 ± 0.06	2.42
Glutamic acid	11.94	13.81	13.50	13.25	13.12 ± 0.41	6.25
Proline	2.55	3.56	3.40	3.37	3.22 ± 0.23	14.16
Glycine	6.25	5.16	5.00	5.54	5.49 ± 0.28	10.15
Alanine	8.43	8.14	8.30	8.26	8.28 ± 0.06	1.57
Cystine	0.02	0.40	0.02	0.23	0.17 ± 0.18	105.88
Valine	6.35	6.03	6.15	6.18	6.18 ± 0.06	2.10
Methionine	1.80	2.02	1.61	1.60	1.76 ± 0.10	11.36
Isoleucine	6.12	5.81	5.75	5.81	5.87 ± 0.09	2.90
Leucine	9.75	9.16	9.20	9.47	9.39 ± 0.14	2.98
Tyrosine	4.27	4.31	4.04	4.02	4.16 ± 0.07	3.60
Phenylalanine	4.94	5.18	4.32	4.34	4.70 ± 0.21	9.15
			g/100 g dry weight			
Protein[c]	46.39	51.68	42.64	43.77	46.09 ± 2.01	8.74
Nitrogen	9.76	10.60	8.97	9.00	9.58 ± 0.39	8.05

[a] Average ± 1 standard error.
[b] Coefficient of variation.
[c] Sum of amino acids.

samples were generally less than the differences which were observed between the various species (Table 1).

Data for the nitrogen content of a number of samples of *Spirogyra* spp., *Pithophora kewensis*, and *Chara* spp. are summarized in Table 3. Average values were similar for the three genera, but there was a wide range in values for different samples of a particular genus.

Table 3. The nitrogen content of dried samples of three genera of algae. Samples were taken from natural populations in different habitats.

Algae	No. of samples	% Nitrogen minimum	$\bar{x} \pm$ S. E.[a]	maximum
Spirogyra spp.	14	0.89	2.69 ± 0.25	4.20
Chara spp.	20	0.64	2.57 ± 0.17	3.80
Pithophora kewensis	16	2.02	2.99 ± 0.22	5.19

[a] Average ± 1 standard error.

Discussion

Boyd (1970) determined the amino acid composition of 11 species of higher aquatic plants. Lysine, alanine, and cystine were more abundant in the protein of algae than in the protein of higher aquatic plants. Algae and higher aquatic plants contained comparable proportions of other amino acids.

The protein content of 11 species of higher aquatics ranged from 4.0 to 21.6 g/100 dry wt. (Boyd 1970). *Euglena proxima* and the three planktonic cyanophyceans contained more protein than higher plants, but the other algae had protein values which were within the range observed for higher aquatic plants. Since the nitrogen content of algae allows an approximation of protein content, nitrogen values from previous studies were compiled in Table 4. Nitrogen levels ranged from 1.77 g/100 g dry wt. in *Mougeotia* to 10.05 g/100 g dry wt. in *Aphanizomenon*, which is essentially the same range of values in Table 1. Phytoplankton apparently contains much more protein than macrophytic algae, but there are large differences in the protein content of various species of either type of algal material.

Samples of the same species of algae from different habitats had wide differences in nitrogen content. These differences are in some cases related to the age of the algae in the different samples since nitrogen levels in plants usually decline with increasing maturity (Boyd 1970). Furthermore, environmental conditions, especially the supply of nutrients, greatly influence the nitrogen content of algae (Gerloff & Skoog, 1954; Spoehr & Milner 1949).

Most algae had relatively similar proportions of the individual amino acids in their protein and nitrogen content may be used to estimate protein.

Therefore, for most ecological purposes, the determination of nitrogen content should allow an adequate assessment of protein levels. The ecological significance of differences in the quantities of protein in plant materials was discussed earlier (BOYD & GOODYEAR 1971), but a few additional comments regarding algae are in order. When compared with protein, there are relatively small differences in the carbon and caloric content of the

Table 4. The nitrogen content of various genera of algae. Samples were collected from natural pupulations.

Genus	No. of samples	Nitrogen (g/100 g dry wt.)	Reference
Microcystis[a, b]	4	8.67	BIRGE & JUDAY (1922)
Microcystis[a, b]	7	6.82	GERLOFF & SKOOG (1954)
Aphanizomenon[a, b]	1	9.77	BIRGE & JUDAY (1922)
Aphanizomenon[a, b]	1	10.05	PRESCOTT (1960)
Anabaena[a, b]	1	8.91	BIRGE & JUDAY (1922)
Anabaena[a, b]	1	9.69	PRESCOTT (1960)
Lyngbya[a, b]	2	5.42	BIRGE & JUDAY (1922)
Oscillatoria[a, b]	1	7.40	TURNER (1916)
Scenedesmus[a]	—	6.68	COOK (1962)
Fragilaria[a]	3	5.41	SCHUETTE (1918)
Volvox[a]	1	6.28	BIRGE & JUDAY (1922)
Cladophora	1	3.77	BIRGE & JUDAY (1922)
Cladophora	1	2.91	SCHUETTE & HOFFMANN (1921)
Rhizoclonium	14	3.16	BOYD & LAWRENCE (1966)
Mougeotia	2	1.77	BOYD & LAWRENCE (1966)
Oedogonium	3	2.64	BOYD & LAWRENCE (1966)
Nitella	3	2.70	BOYD & LAWRENCE (1966)

[a] Plankton algae.

[b] Cyanophyta.

organic matter of samples of various species of dehydrated algae (BOYD & LAWRENCE 1966; CUMMINS & WUYCHECK 1971). A herbivore feeding on *Spirogyra* sp. would consume about the same number of calories as a second herbivore feeding on *Euglena proxima*. However, the animal feeding on *E. proxima* would consume roughly three times as much protein per unit of food consumed as the animal feeding on *Spirogyra* sp. Furthermore, the decomposition of plant materials with a high protein content is much faster than for materials with a low protein content (ALEXANDER 1961) and phytoplankton will decompose much faster than macrophytic algae.

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Summary

The protein content of 13 species of freshwater algae ranged from 10.50 to 46.35 g/100 g dry wt. Protein levels in planktonic cyanophyceans and in *Euglena proxima* were above 40 g/100 g dry wt, but macrophytic chlorophyceans and one planktonic chlorophycean, *Dictyosphaerium pulchellum*, contained 20.25 g/100 g dry wt or less protein. In most instances, the proportion of the protein comprised by a particular amino acid was similar in the different species.

The nitrogen content of algae was a good indicator of protein content. Samples of the same genus or species from different habitats had wide differences in nitrogen content. Differences in the protein content of different populations of algae will influence the efficiency with which these food materials are utilized in food webs.

Zusammenfassung

Der Eiweißgehalt von 13 Süßwasser-Algenarten lag zwischen 10,50 und 46,35 g/100 g Trockensubstanz. Die Eiweißmengen lagen in planktonischen Cyanophyten und in *Euglena proxima* über 40 g/100 g Trockensubstanz. Makrophytische Chlorophyten und eine planktische Chlorophycee, *Dictyosphaerium pulchellum*, enthielten nur 20,25 g/100 g Trockensubstanz oder noch weniger Protein. In einigen Fällen ähnelte sich die durch eine besondere Aimnosäure gekennzeichnete Eiweißzusammensetzung.

Der Stickstoffgehalt der Algen stellte einen guten Indikator des Eiweißgehaltes dar. Jedoch unterschieden sich Algen der gleichen Gattung oder auch Arten von verschiedenen Biotopen stark im Stickstoffgehalt. Unterschiede im Eiweißgehalt der verschiedenen Algenpopulationen wirken sich natürlich auf die Effektivität dieser Nahrungsstoffe im Nahrungskreislauf aus.

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