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GENERAL CHARACTERISTICS OF THE POLYSACCHARIDES OF BROWN ALGAE

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It has been shown previously [1, 2] that Ascophyllum nodosum and Laminaria hyperborea (brown algae) contain acid polysaccharides with a firmly bound protein component. Until now there has been no information on the presence of mixed carbohydrate-protein compounds in other brown algae.

Table 1

Algae	Content, %								
	alginic acid (fr. 1)			other polysaccharides (fr. 2)			protein in the polysaccharides (fr. 3)		
	spring	summer	autumn	spring	summer	autumn	spring	summer	autumn
Sargassum pallidum (I)	22.5	34.3	40.1	8.0	10.0	23.2	8.1	23.0	24.4
Laminaria japonica (II)	26.1	37.2	54.3	13.7	15.5	11.7	18.9	4,1	5.9
Chordaria magellanica (III)	12.0	—	23.6	23.8	—	31.7	14.7	—	3.8
Fucus evanescens (IV)	—	—	22.7	—	—	33,8	—	—	21.2
Desmarestia viridis (V)	8.8	19.2	9.5	10.0	11.0	13.5	5.3	7.1	10.2
Dictiota sp. (VI)	10.0	—	26.0	16.7	—	10.0	3.3	—	13.1
Cystoseira sp. (VII)	36.0	22.5	—	14.8	15.0	—	—	5.3	—
Pelvetia sp. (VIII)	41.8	17.5	43.2	18.4	22.0	25.0	18.9	5.0	5.1
Heterochordaria abietina (IX)	—	—	40.8	—	—	24.2	—	—	12.7
Costaria costata (X)	33.4	39.3	—	8.0	21.4	8.8	2.0	—	—
Scytosiphon sp. (XI)	36.6	—	—	13.0	—	—	5.3	—	—
Laminaria Cicharioides (XII)	30.0	34.4	49.6	8.0	7.9	30.2	5.0	12.0	6.3
Sphaerotrichia sp. (XIII)	—	—	19.6	—	—	40.0	—	—	13.2

We have studied the brown algae of the Sea of Japan collected in 1966-1967 during various vegetation periods. Three samples of each species were studied in parallel. Table 1 gives the yield of alginic acid (fraction 1) and the content of polysaccharide fraction after the elimination of the alginic acid by acidification (fraction 2). Fraction 2 was treated by Sevag's method to give fraction 3. The protein content in this fraction varied from 2.0-24%. However, it gave no precipitate with trichloroacetic acid and sodium tungstate, which may show the complete precipitation of the uncombined protein.

Table 2 shows the monosaccharide and amino acid composition of the 3rd fraction from the brown algae. It can be seen from the table that for the majority of algae this fraction has qualitatively similar monosaccharide and amino acid compositions. A characteristic feature is the presence of large amounts of galactose, fucose, and xylose. Uronic acids are present in fraction 3 from all the algae studied, but their amount (from 5.0 to 25%) is considerably lower than the content of neutral monosaccharides. The peptide component is made up of a fairly large selection of ordinary amino acids and only in some cases were the spots of unidentified amino acids with low R_f values found on chromatograms.

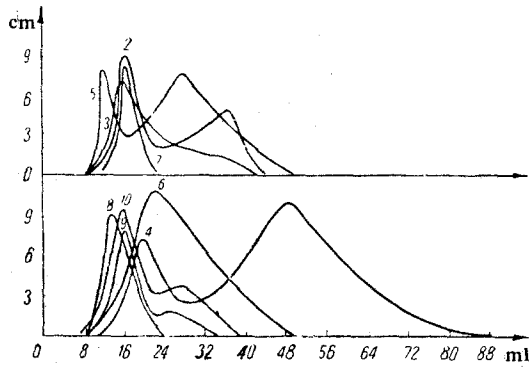

Fig. 1. Gel filtration of brown algae polysaccharides on Biogel P-20: 1) Sargassum pallidum; 2) Laminaria japonica; 3) Chordaria magellanica; 4) Fucus evanescens; 5) Desmarestia viridis; 6) Dictiota sp.; 7) Pelvetia sp.; 8) Heterochordaria abietina; 9) Laminaria cicharioides; 10) Sphaerotrichia sp.

To elucidate the distribution of the component of fraction 3 with respect to molecular weights, gel filtration was carried out on Biogels P-20 and P-60 (Figs. 1 and 2). In a number of cases, only one peak was obtained on the elution curves but generally there were two, which may show the lack of homogeneity of fraction 3. The course of gel filtration was checked automatically by means of a continuous ultraviolet densitometer and in parallel by the phenol-sulphuric acid method [4]. In addition, the eluates were analyzed on the basis of a protein content determination by Lowry's method (fractions from the column) [5]. Analyses by all three methods gave curves of similar shape, which confirms the relationship between the polysaccharide and the peptide components in fraction 3.

Table 2

Algae	Monosaccharide composition	Amino acid composition																
		Cys	Lys	His	Arg	Asp	Ser	Gly	Thr	Glu	Ala	Tyr	Met	Val	Pro	Phe	Leu	Ileu	N
(I)	Gal* Xy* Fu* Man Galur Glur	+	+	+	+	+	+	+	+	+	+		+	+		+	+	+	+
(II)	Gal* Xy* Fu* Gl Man Galur	+	+	+	+	+	+	+	+	+	+	+?	+?	+		+	+	+	
(III)	Gal* Xy* Fu* Gl Man? Galur	+	+	+	+	+	+	+	+	+		+?		+		+	+	+	+
(IV)	Gal* Xy Fu* Man *Glur	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	
(V)	Gal* Xy* Fu Man Galur Glur	+	+	+	+	+	+	+	+	+	+	+		+		+	+	+	
(VI)	Gal* Xy* Fu Gl Man* Glur*	+	+	+	+	+	+	+	+	+	+		+	+		+	+	+	
(VII)	Gal* Xy* Fu* Gl* Man Glur	+	+	+	+	+	+	+	+	+	+	+		+		+	+	+	+
(VIII)	Gal* Xy* Fu* Gl Man Galur Glur	+	+	+	+	+	+	+	+	+	+?	+		+		+	+	+	+
(IX)	Gal* Xy* Fu* Gl Man Galur Glur				+	+	+	+	+	+		+	+	+		+	+	+	
(X)	Gal* Xy* Fu* Gl Man Rha	+	+	+	+	+	+	+	+	+	+	+		+		+	+	+	
(XI)	Gal* Xy* Fu* Gl* Man? Galur*	+	+	+	+	+	+	+	+	+	+	+		+		+	+	+	+
(XII)	Gal* Xy* Fu* Gl* Man Galur Glur	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
(XIII)	Gal* Xy* Fu* Gl Galur Glur	+	+			+	+	+	+	+	+	+	+	+		+	+	+	

Notes. Gal) galactose; Gl) glucose; Xy) xylose; Man) mannose; Fu) fucose; Rha) rhamnose; Galur) galacturonic acid; Glur) glucuronic acid + glucuronolactone; N) unidentified amino acids; *) main monosaccharide component.

Experimental

Chromatography was carried out on Whatman No. 3 paper with the following systems of solvents (by volume): 1) ethyl acetate–pyridine–glacial acetic acid–water (5 : 5 : 1 : 3); 2) glacial acetic acid–ethyl acetate–water (1 : 2 : 3), and 3) butan-1-ol–glacial acetic acid–water (4 : 1 : 1). The spots were revealed with the following reagents: 1 and 2–aniline hydrogen phthalate; 3–0.2% solution of ninhydrin in acetone. The content of uronic acids in the polysaccharide fractions was determined by the carbazole method [6] and the content of proteins colorimetrically by Lowry's method [5]. All the solutions were evaporated in vacuum at 30–50° C. Gel filtration was done using Biogels of the firm Bio Rad Laboratories (California, USA).

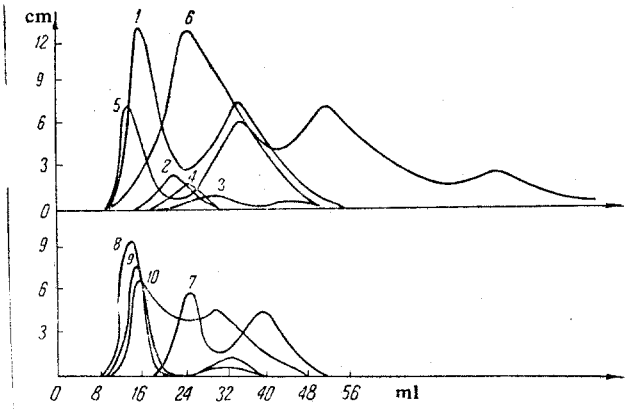

Fig. 2. Gel filtration of brown algae polysaccharides on Biogel P-60. 1) Sargassum pallidum; 2) Laminaria japonica; 3) Chordaria magellanica; 4) Fucus evanescens; 5) Desmarestia viridis; 6) Dictiota sp.; 7) Pelvetia sp.; 8) Heterochordaria abietina; 9) Laminaria cicharioides; 10) Sphaerotrichia sp.

Isolation of the total polysaccharide fraction. Fiftyy grams of fresh algae was extracted with methanol in Soxhlet apparatuses. The residue was dried to constant weight. The standard defatted sample was subsequently extracted with dilute acetic acid (pH 5–6) at 50° C and then with distilled water at 90° C, and exhaustively with 0.5% ammonium oxalate solution at 75° C. The alginic acid (fraction 1) precipitated from the combined solution on acidification to pH 1.5–2. This was separated by centrifuging, dried in a vacuum desiccator, and the yield was determined gravimetrically (see Table 1). The centrifugate was neutralized with sodium carbonate solution, dialyzed against distilled water for 2 hr, and evaporated to small bulk. The polysaccharides were precipitated with a fourfold volume of methanol. The precipitate of polysaccharides (fraction 2) was filtered off, dried, and weighed, the yields being given in Table 1. A mixture of 1 g of polysaccharides (fraction 2), 100 ml of water, 25 ml of chloroform, and 10 ml of amyl alcohol was shaken for 1 hr; the chloroform layer was separated off and the polysaccharide fraction was precipitated with methanol. This treatment was repeated not less than twice, giving fraction 3, which we are still studying.

Acid hydrolysis of the polysaccharide fraction. Determination of the monosaccharide composition. Twenty milligrams of the polysaccharides was hydrolyzed with 1 ml of 2 N sulfuric acid at 100° C for 10 hr. The reaction mixture was filtered, neutralized with barium carbonate, and treated with Amberlite IR-120. Chromatography was performed in system 1 to separate the uronic acids and in system 2 to separate the fucose and xylose (see Table 2).

Determination of the amino acid composition. Ten milligrams of polysaccharides was heated with 1 ml of 6 N hydrochloric acid at 105° C for 20 hr. The hydrolysate was evaporated in a vacuum desiccator over caustic potash, moistened with water, and again evaporated to dryness. This operation was repeated twice more. The residue was chromatographed in system 3 as described previously [7].

Conclusions

It has been shown that the brown algae of the Sea of Japan contain, in addition to alginic acid, acid polysaccharides probably bound to a peptide component.

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