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MOLECULAR CHARACTERISTICS OF SUBFRACTIONS OF LAMINARIN

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At the present time, the traditional idea of laminarins from algae as linear β-1,3-glycans with different numbers of β-1,6-glycosidic bonds in the form of singe branches is changing. Thus, a laminarin has been found (from *Eisenia bicycles*) with a block structure in which β-1,6-bound residues are included in a β-1,3-glucan chain [1]. The laminarin of *Chorda filum* has a different lengths of sequences of β-1,6-bound glucose residues included in the main chain [2]. Finally, a laminarin from *Emiliama huxleyi* was been ascribed a completely different structure: β-1,6- blocks contain β-1,3-attached branches in the form of gentiobiose [3]. The present work has shown some variety of the structures obtained by the narrow fractionation of a laminarin from the alga *Laminaria cichirioides*.

A sample of the laminarin (**1d**) was dissolved in 40 ml of distilled water, the solution was centrifuged at 5000 rpm for 45 min, and the insoluble part (1.9%) was removed. Dioxane was added dropwise to the solution until a persistent turbidity had appeared, after which the mixture was heated to 32°C and was then cooled to 20°C and centrifuged at 3000 rpm for 40 min. A series of fractions was isolated, the last one being obtained by evaporating the solution completely (Table 1). The fractions were investigated by the method of molecular hydrodynamics [4] in 0.1 M NaCl at 25°C. The characteristic viscosity $[\eta]$ and the coefficient of translational diffusion D (on a polarization diffusiometer [4]) were determined. From the values of

TABLE 1. Characteristics of the Laminarin Subfractions Obtained

Fraction No.	$W_i \times 10^2$	V_{pr}/V_s	$[\eta]$, cm^3/g	$D \times 10^7$, cm^2/s	$M_{D\eta} \times 10^{-3}$	β-1,3/β-1,6*
1	3.76	0.48	–	–	–	16
2	7.73	1.99	–	–	–	12
3	23.13	2.56	8.5	14.0	8.9	9
4	13.26	2.71	8.1	13.4	10.6	8.7
5	17.61	3.03	7.9	14.8	8.1	7.7
6	14.33	4.06	6.4	16.7	7.0	5.1
7	10.41	7.59	4.3	23.2	3.9	7.6
8	8.77	–	–	–	–	13.6

W_i is the mass proportion of the fraction; V_{pr} and V_s are the volumes of the precipitant and of the solution

*Results of the ^1H NMR spectra; (—) — no determination made.

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TABLE 2. Results of Methylation by Hakomori's Method [6]

Fraction No.	Glucose residues, %				Presence of mannitol	Calculated		
	terminal	1,3	1,6	1, 3, 6		reduc. ends %	degree of polymerization	
							1,3	1,6
2	9.8	82	–	7.6	–	2.2	45	11.7
5	15.9	73	4.2	6.9	+	9.0	11	7.3
Initial laminarin	13.3	74.5	2.2	8	2%	5.3	19	6.1

[η] and D, using the theoretical value of the hydrodynamic invariant $A_0 = 3.84 \times 10^{-10}$ erg/deg·mole$^{1/3}$ we calculated their molecular masses M from the formula

$$M_{D\eta} = \left(\frac{A_0 \cdot T}{\eta_o}\right) \cdot \left(D^3 \ [\eta_o]\right)^{-1},$$

where η_0 is the viscosity of the solvent. Table 1 shows a tendency to a decrease in molecular mass with an increase in the fraction number.

NMR spectroscopy (Bruker WM-250) showed that fractionation had taken place not only with respect to mass but also with respect to composition. The PMR spectra of the fractions had different ratios of the integral intensities of the signals of the anomeric protons of β-1,3- (the 4.70-5.30 ppm region) and β-1,6- (4,40-4.60 ppm) glycosidic bonds, the nature of the signals showing different distributions of the β-1,6-bound residues along the polymeric chain [5]. For fractions 1, 7, and 8, in the spectra of which the signals of anomeric protons of a reducing monosaccharide residue were observed, we evaluated the degrees of polymerization ($\sim$13, 22, and 7, respectively). The ^{13}C NMR spectra showed that samples 3, 4, and 5 contained mannitol residues (δ = 64.4 ppm; 3%, 4%, and traces, respectively). These differences were also confirmed by the results of methylation (Table 2).

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