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BRIEF COMMUNICATIONS

COMPOSITIONAL HETEROGENEITY OF SULFATED POLYSACCHARIDES SYNTHESIZED BY THE BROWN ALGA *Costaria costata*

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Brown algae contain sulfated polysaccharides, fucoidans, that exhibit various biological activities [1]. The variety of activities exhibited by fucoidans is related to their structural variations. It was shown that species of a single family (genus) of brown algae can contain sulfated polysaccharides that differ in structure and biological activity [2]. Correspondingly, several different fractions of fucoidans can be isolated from a single species [3, 4]. Our goal was to determine the composition of sulfated polysaccharides synthesized by the brown alga *Costaria costata* [Turn.] Saund (Laminariaceae), which is broadly distributed in seas of the Russian Far East.

Polysaccharides were isolated by acid extraction at room temperature [5] from specimens of *C. costata* collected in July in Troits Bay (Sea of Japan). The polysaccharide fraction was separated into fucoidan (F) and laminaran by chromatography over the hydrophobic sorbent Polikhrom-1 [5]. The monosaccharide composition of the acid-hydrolysis products of the polysaccharides was determined by HPLC in an IC-5000 Biotronik carbohydrate analyzer (Shim-pack ISA-07/S2504 column, 0.4 × 25 cm, potassium borate buffer, flow rate 0.6 mL/min). Monosaccharides were detected using the bicinchoninate method. Monosaccharides (Rha, Man, Fuc, Gal, Xyl, Glc) were used as standards. The molecular weights (MWs) of the polysaccharides were determined by HPLC in a Shimadzu LC-20A instrument with an RID-10A refractometric detector. Fucoidan samples were dissolved in doubly distilled water and filtered through a membrane filter (0.45 μm pore size). Fucoidans were separated over successively connected columns of Shodex Asahipak GS-520 HQ and GS-620 HQ (7.5 × 300 mm) at 50°C with elution by H_2O (0.8 mL/min). Columns were calibrated using standard pullulans of MWs from 180 to 667,000 Da (Polymer Laboratories, USA) and blue dextran (Amersham, Sweden).

Analysis of the monosaccharide composition of fraction F showed that fucose and galactose were the major monomers. Mannose, rhamnose, xylose, and glucose were detected in smaller quantities. The MW distribution of fraction F was heterogeneous. Three maxima with the strongest at 300 kDa and weaker ones at 80 and 560 were observed in the MW distribution curve.

Fucoidan from *C. costata* collected in July had the following characteristics: yield 2.5% (% of dry alga); uronic acids, 2.2% (of fraction weight); protein, 3.1% (of fraction weight); SO_3Na^-, 17.3% (of fraction weight); monosaccharides (mol%): Fuc, 55.1; Gal, 18.1; Man, 9.2; Rha, 11.5; Xyl, 4.3; Glc, 1.8. Protein was determined by the Lowry method [6]; sulfates, by turbidimetry [7]; uronic acids, spectrophotometrically [8]. Fucoidan (F) was also fractionated by ion-exchange chromatography over DEAE-cellulose (3.5 × 14 cm). Retained polysaccharides were eluted by a linear gradient of H_2O–NaCl (2 M) to afford fractions F-0.5 and F-1.5 (Table 1).

Fraction F-0.5 contained a low-sulfated fucoidan with a heterogeneous monosaccharide composition and a high content of mannose and uronic acids (37 and 15.5%, respectively, of total monosaccharides) (Table 1). Uronic acid in F-0.5 and F-1.5 was identified after total hydrolysis as glucuronic acid using GC of the polyol acetates [9]. This fraction was characterized as uronofucomannans according to the monosaccharide composition determined using GC of the polyol acetates [9].

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Table 1. Characteristics of Fractions F-0.5 and F-1.5 Obtained by DEAE-Cellulose Chromatography of Fucoidan from *C. costata*

Fraction	Yield,%**	MW_{av}, kDa	SO_3Na^-,%*	Monosaccharides, mol%						
				Fuc	Gal	Man	Rha	Xyl	Glc	GlcA
F-0.5	29	80, 620	6.7	22.6	6.4	37.0	4.2	4.6	9.6	15.5
F-1.5	69	300, 80	23.8	70.2	19.8	7.0	0.0	0.0	0.0	3.0

*% of fucoidan fraction weight; **% of initial fraction.

The MW distribution of F-0.5 was heterogeneous. It contained in approximately equal amounts fucoidan of average MW (MW_{av}) 80 kDa and 620 kDa.

Fraction F-1.5 consisted of about 70% of the initial F and had a simpler monosaccharide composition than F-0.5. Fucose and galactose (1.0:0.28 ratio) accounted for about 90% of the total monosaccharides. The fraction had a high content of sulfates (Table 1). It was identified as a galactofucan according to the monosaccharide composition. The MW distribution of F-1.5 was also heterogeneous. The principal part consisted of fucoidans with MW_{av} 300 kDa with a smaller fraction with MW_{av} 80 kDa.

Thus, F isolated from *C. costata* collected in July contained as a minimum two groups of fucose-containing polysaccharides with different structures in a 1:2 ratio. These were low-sulfated uronofucomannan with maxima on the MW distribution at about 80 and 600 kDa and high-sulfated galactofucan with MW_{av} 300 kDa. The study of the structural characteristics of F fractions isolated from *C. costata* collected in July is continuing.

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