A Novel Antivirally Active Fucan Sulfate Derived from an Edible Brown Alga, Sargassum horneri

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A novel fucan sulfate (Hor-1) was isolated from the hot water extract of an edible brown alga, Sargassum horneri (Turner) C. AGARDH. The fucan sulfate was revealed to have sugar linkage types, sulfate content and uronic acid content different from those of sodium hornan (Na-HOR), another fucan sulfate isolated from this alga. However, it exhibited inhibitory activity against replication of herpes simplex virus type 1 with similar potency to Na-HOR.

Key words fucan sulfate; antiviral activity; Sargassum horneri; brown alga; sulfated polysaccharide

In East Asia, marine algae have been used as rich sources of minerals, vitamins and dietary fibers. They have been highlighted recently as multifunctional foods for maintaining our health. Sargassum horneri (Turner) C. AGARDH is a Sargassaceae brown alga and is distributed over the shallow sea of Japan, except for in Hokkaido. The slightly boiled alga has been used as a savory food in several regions along the Japan Sea. In the previous study, we isolated a fucan sulfate named sodium hornan (Na-HOR) from the hot water extract of this alga, collected at Notojima in Ishikawa Prefecture, and showed that Na-HOR exerted potent antiviral activity in vitro. So far, little study of the regional difference of sulfate polysaccharides in algae has been performed. In the continuing study, we isolated a new fucan sulfate from the same alga collected at Yamada Bay in Iwate Prefecture. This paper describes the results of the structure determination of the fucan sulfate as well as an evaluation of its antiviral effects.

Experimental

Materials S. horneri was collected at Yamada Bay in Iwate Prefecture, Japan in May, 1999. A standard molecular weight marker of pullulans (Shodex Standard P-82) was purchased from Showa Denko Co., Ltd., (Japan) and dextran sulfate sodium salt (MW 50000) was from Sigma-Aldrich Japan KK.

General Experimental Procedures Optical rotation was determined in H2O using a 1 cm path length cell with a JASCO DIP-1000 digital polarimeter. UV absorptions were measured with a JASCO V-530 UV/VIS spectrophotometer. IR spectra were recorded in a KBr disk or liquid film using a Hitachi 260-10 IR spectrophotometer. Metall elements and sulfur were analyzed with a Hitachi scanning electron microanalyser X-650. Carbohydrate content was determined by phenol-H2SO4 method. HPLC, GC and GC-MS analysis were performed as described previously.

Extraction and Fractionation of Polysaccharide Fresh algal material (2.4 kg) was cut into pieces and extracted twice with 2 l of boiling water for 1 h. After the hot water extract was concentrated, the extract was treated with 80% EtOH. The resulting precipitate was filtered, washed with cold ETOH and dried in a desicator to give a brownish residue (H). Yield: 102.9 g.

Gel Filtration of H H (1.5 g) was dissolved in 0.01 M citrate buffer, pH 7.0, containing 0.1 M NaCl, and the soluble portion was applied to a column of Sepharose 6B (Pharmacia Biotech, Uppsala, Sweden, 4.4×70 cm). The column was eluted with the same buffer, and fractions of 10 ml were collected. The eluted fractions were separated into two fractions (H-1, H-2) according to the elution profile prepared on the basis of the phenol-H2SO4 method at 480 nm and UV absorbance at 260 nm. Yield: H-1, 246 mg; H-2, 295 mg.

Isolation of Sulfated Polysaccharides from H-1 H-1 (246 mg) dissolved in H2O was applied onto a column of DEAE Toyopearl 650M (Toosoh, Tokyo, Japan, 5×17 cm). The column was eluted successively with 100 ml of 0.5 M NaCl and 400 ml of a linear gradient of 0.5—1.5 M NaCl, and fractions of 10 ml were collected. The eluted fractions were separated into two fractions (H-1a, H-1b). H-1b was concentrated, dialyzed against H2O and lyophilized to yield colorless residue (121 mg). The residue was further purified with an ion exchange column on DEAE Toyopearl 650M in the same conditions as described above. The main peak was collected, dialyzed, lyophilized and subjected to rechromatography with a sepharose 6B column (2×60 cm) to give a colorless polysaccharide Hor-1 (24 mg).

Characterization and Sugar Linkage Determination of Hor-1 The estimation of molecular weight, identification of component sugars, and sugar linkage determination by methylation analysis were performed as described in the previous paper. Electrophoresis was performed on a cellulose acetate membrane (Separax) using veronal buffer (pH 8.6, I=0.06). The quantity of sulfate and uronic acid in Hor-1 were determined according to the methods reported by Silvestri et al. and Blumenkranz and Asboe-Hansen, respectively.

Cytotoxicity and Antiviral Assay The cytotoxicity (CC50) and anti-herpes simplex virus type 1 (HSV-1) activity (IC50) was measured as described in the previous paper. The potency of anti-HSV-1 activity was evaluated by calculation of the selectivity index (CC50/IC50).

Results and Discussion

The hot H2O extract from the fresh algal fronds was treated with 80% EtOH and the precipitate was then dialyzed against running water. The non-dialyzate gave an acidic polysaccharide, Hor-1, by fractionation with gel filtration on Sepharose 6B and ion exchange column chromatography on DEAE Toyopearl 650M. As indicated in Fig. 1, Hor-1 was detected as a single band in the electrophoresis. Therefore, Hor-1 was suggested to be a homogeneous polysaccharide on the basis of molecular size and charge distribution. The apparent molecular weight of Hor-1 was estimated to be 2.7×10^5. It was shown that fucose was the sole component sugar in Hor-1 by sugar composition analysis. Little uronic acid was detected in Hor-1 (<1%) in contrast with Na-HOR (4%). The negative optical rotation of Hor-1 (−114.8°) indicated that it consisted of α-L-fucose. X-ray microanalysis suggested the presence of sodium and sulfur in the molecule. The observation of S=O stretching band at 1250 cm⁻¹ in the IR spectrum indicated that the polysaccharide is a fucan sulfate. The sulfate contents of Hor-1 was 17.0%, and its degree of sulfate substitution (D.S.) was calculated as 0.4 mol per anhydro fucose residue. This value indicated that Hor-1 was more highly sulfated than Na-HOR (D.S. 0.26).

The linkage modes and the positions of the sulfate groups in Hor-1 were determined by methylation analysis of the triethylamine salt (TEA-Hor-1) and desulfated polysaccharide.
Although methylation analysis of sulfated polysaccharide is not quantitative, it may offer valuable information concerning the position of the glycosidic linkage and the site of sulfation. As summarized in Table 1, it was suggested that Hor-1 was a highly branched polysaccharide, since large amounts of mono- and unmethylated fucose derivatives were identified in DS-Hor-1. About one-fifth of the fucose residue was estimated to be branched residue. Other fucose derivatives revealed the presence of various linkages such as 1,2-, 1,3- and 1,4-linkages. By comparing the result of the methylation analysis of TEA-Hor-1 with that of DS-Hor-1, sulfate groups were found to be substituted at C-2 or C-4 in 1,3-linked residue and C-3 of 1,2- and 1,4-linked residues. In addition, the presence of disulfated 1,4-linked residue was also suggested.

When Hor-1 was evaluated for antiviral activity on the basis of the selectivity index (CC50/IC50), it was found to be a potent inhibitor of the replication of HSV-1. That is, the selectivity index of Hor-1 was 11000 when it was added to the medium at the same time as the viral infection and throughout the incubation thereafter, and 7100 when it was added to the medium immediately after the viral infection. Its potency was similar to that of Na-HOR.

The present study indicated structural diversity of sulfated polysaccharides produced by an alga growing at different places. The seasonal variation of the active principle is currently under investigation.

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