Carbohydrate Polymers 86 (2011) 995–999

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Structures and antiviral activities of polysaccharides from *Sargassum trichophyllum*

Jung-Bum Lee*, Azumi Takeshita, Kyoko Hayashi, Toshimitsu Hayashi

Graduate School of Medicine and Pharmaceutical Sciences for Research, University of Toyama, 2630 Sugitani, Toyama, Toyama 930-0194, Japan

ARTICLE INFO

Article history:
Received 27 July 2010
Received in revised form 23 May 2011
Accepted 26 May 2011
Available online 6 June 2011

Keywords:
Sargassum trichophyllum
Laminaran
Alginate
Fucoidan
Antiviral activity

ABSTRACT

Isolation and characterization of polysaccharides from *Sargassum trichophyllum*, a type of brown algae, were performed. Three polysaccharides, ST-L, -A and -F were obtained and characterized as laminaran, alginate and fucoidan, respectively. ST-L was found to be a β-glucan consisting of terminal- (14.5 mol%), 1,3- (69.4%), 1,6- (33.4%), and 1,3,6-linked (12.8%) residues. ST-A was found to be a mannuronic acid-rich alginate (M/G = 1.88). ST-F consisted of fucose (79.1 mol%) and galactose (19.9 mol%), and its sulfate content was estimated to be 25.5%. Methylation analysis revealed that ST-F was mainly composed of terminal, 1,4- and 1,3-linked fucose and terminal, 1,2- and 1,6-linked galactose residues. Among these polysaccharides, only ST-F showed antiviral activity against herpes simplex virus type 2.

1. Introduction

In East Asia, some of the marine macroalgae (seaweeds) are familiar foodstuffs and have also been used as crude drugs. They produce various metabolites and have been recognized as promising targets for discovering bioactive substances. In particular, marine macroalgae produce a variety of polysaccharides, which have been attracting attention as pharmaceuticals, food ingredients and cosmetic ingredients. Among them, laminarans, alginates and fucoidans are polysaccharides produced by brown algae. Laminarans are water-soluble storage polysaccharides consisting of β-1,3- and β-1,6-linked D-glucose residues. Their structures are known to vary considerably depending on the source (Chizhov et al., 1998; Shevchenko et al., 2007; Usov & Chizhov, 1993). Alginates are important phycocolloids, which consist of α-L-gluronic acid and β-D-mannuronic acid. These polymers have been used for decades as thickeners, stabilizers and gelling agents in the food and pharmaceutical industries. Fucoidans are fucose-containing sulfated polysaccharides obtained from brown seaweeds. They are heterogeneous assemblages with regard to their molecular mass, monosaccharide composition and number of sulfate and acetyl groups. On the other hand, fucoidans are well known to possess various biological activities such as antitumor, antivirus and anticoagulant activities (Berteau & Mulloy, 2003; Ghosh et al., 2009; Kusaykin et al., 2008).

Sargassum trichophyllum belongs to Sargassaceae and distributes along the sea shore of Japan. *Sargassum* sp. has been recorded as a crude drug in oriental medical dictionaries for treating goitre, scrofula, and testicular pain and swelling. In addition, *Sargassum* is one of the most familiar seaweed in Japan and some of them have been used as food. So far, we have reported that the structures and antiviral effects of fucan sulfates (fucoidans) from *S. horneri* (Hoshino et al., 1998; Preeprame, Hayashi, Lee, Sankawa, & Hayashi, 2001). However, these results revealed that there are structural diversity and differences in antiviral potencies among them. In addition, our comparative research on the biological activities of polysaccharides from algae also revealed that the antiviral effects seemed to be correlated with not only sulfate contents and molecular weights but also sugar composition and/or their linkage types (Hayakawa et al., 2000; Lee, Hayashi, Maeda, & Hayashi, 2004). Therefore, we have been interested in the correlation between sugar composition and antiviral effects of sulfated polysaccharides, and this prompted us to characterize and evaluate the structures and biological activities of polysaccharides from various algae. During the course of the study, we collected *S. trichophyllum* from Noto Island in Japan. So far, there is no report about the chemical constituents including polysaccharides of this alga. Here, we report the results of characterization and evaluation of antiviral effects of polysaccharides from *S. trichophyllum*.

* Corresponding author at: Laboratory of Phamacognosy, Graduate School of Medicine and Pharmaceutical Sciences for Research, University of Toyama, 2630 Sugitani, Toyama, Toyama 930-0194, Japan. Tel.: +81 76 434 7580; fax: +81 76 434 5170.
E-mail address: lee@pha.u-toyama.ac.jp (J.-B. Lee).

0144-8617/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2011.05.059

2. Materials and methods

2.1. Materials

S. trichophyllum (gametophyte) was collected from the shores of Noto Island in Japan in May, 2006. A voucher specimen was deposited in the Laboratory of Pharmacognosy, Graduate School of Medicine and Pharmaceutical Sciences for Research, University of Toyama (Japan).

2.2. Isolation of polysaccharides from S. trichophyllum

Seaweed (600 g) was cut into pieces and extracted with H_2O for 1 h under reflux (three times). The combined extract was concentrated *in vacuo* and lyophilized to give a brownish residue (ST, 29 g). Twenty gram of ST was dissolved in H_2O and dialyzed against H_2O. Non-dialyzate and dialyzate were concentrated and lyophilized to give high molecular weight (STH, 5.8 g) and low molecular weight fractions (STLM, 11.2 g), respectively. STH was dissolved in H_2O and applied to a DEAE 650M anion exchange column (5 cm i.d. × 14 cm), which was successively eluted with H_2O, 0.5 M NaCl, and 1 M NaCl to give STH1 (0.8%), STH2 (48.0%), and STH3 (24.4%), respectively. STH1 (94 mg) was dissolved in 0.1 M NaCl and applied to a Sephacryl S-100 HR column (2.5 cm i.d. × 94 cm) and eluted with 0.1 M NaCl. Fractions of 3 ml were collected and monitored by the phenol–H_2SO_4 method (Dubois, Gilles, Hamilton, Revers, & Smith, 1956) and UV detection at 275 nm to monitor colored materials and proteins. Carbohydrate positive fractions were concentrated and lyophilized to give ST-L (88.2 mg). STH2 was dissolved in H_2O and applied to a DEAE 650M anion exchange column (5 cm i.d. × 15 cm), and eluted with a linear gradient system prepared by H_2O and 1 M NaCl. Fractions of 20 ml were collected and monitored by the phenol–H_2SO_4 method and UV detection at 275 nm. STH2a (52%) and 2b (44%) were obtained on the basis of elution profile. STH2a was dissolved in 10 mM citrate buffer (pH 7) containing 0.1 M NaCl and applied to a Sepharose 6B column (4.4 cm i.d. × 94 cm) followed by elution with the same buffer. Fractions of 15 ml were collected and monitored by the phenol–H_2SO_4 method and UV detection at 275 nm, and gave STH2a-1 (10%), -2 (54%) and -3 (11%) on the basis of elution profile. STH2a-2 was applied to a Sephacryl S-300 HR column (2.2 cm i.d. × 94 cm) and eluted with 0.1 M NaCl. Carbohydrate positive fractions were pooled together, dialyzed and lyophilized. This column chromatography was repeated three times and gave ST-A (37%). STH3 was dissolved in H_2O and applied to a DEAE 650M anion exchange column (5 cm i.d. × 15 cm), and eluted with a linear gradient system prepared by H_2O and 1 M NaCl. Fractions of 20 ml were collected and monitored by the phenol–H_2SO_4 method and UV detection at 275 nm. STH3a (17%) and 3b (58%) were obtained on the basis of elution profile. STH3b was dissolved in 10 mM citrate buffer (pH 7) containing 0.1 M NaCl and applied to a Sepharose 6B column (4.4 cm i.d. × 94 cm), which eluted with the same buffer. Fractions of 15 ml were collected and monitored by the phenol–H_2SO_4 method and UV detection at 275 nm, and gave STH3b-1 (19%) and -2 (53%) on the basis of elution profile. STH3b-2 was applied to a Sephacryl S-300 HR column (2.2 cm i.d. × 94 cm) and eluted with 0.1 M NaCl. Carbohydrate positive fractions were pooled together, dialyzed and lyophilized. This column chromatography was repeated twice to give ST-F (57%).

2.3. Estimation of molecular weight

The molecular weight of isolated polysaccharides was estimated by HPLC analysis. The sample was applied on TSK GMPW$_{XL}$ gel filtration columns (7.6 mm × 300 mm × 2; Tosoh, Tokyo, Japan) and eluted with 0.1 M $NaNO_3$ at 0.6 ml/min. Commercially available pullulans (Shodex P-52; Showa Denko K.K., Tokyo, Japan) were used as standard molecular markers.

2.4. Colorimetric analyses of polysaccharides

Uronic acid content was determined by *m*-hydroxydiphenyl method (Blumenkrantz & Asboe-Hansen, 1973). Protein content was determined using a Bio-Rad protein assay kit. Sulfate content was determined by rhodizonate method (Silvestri, Hurst, Simpson, & Settine, 1982).

2.5. Sugar composition and methylation analyses of polysaccharides

Polysaccharides were hydrolyzed with 2 M trifluoroacetic acid at 120 °C for 1 h. After removal of TFA under N_2 gas, the hydrolyzates were converted to alditol acetates, which were analyzed by GC using a SP-2330 fused silica capillary column (30 m × 0.32 mm i.d.; Supelco, MA, USA) with the oven temperature of 200–240 °C (4 °C/min). Desulfation of ST-F was performed by solvolytic desulfation with 10% MeOH/DMSO (Nagasawa, Inoue, & Tokuyasu, 1979). After dialysis and lyophilization, a colorless polysaccharide was obtained (D-ST-F, Yield: 35%). ST-F was converted to the triethylammonium salt (TEA-ST-F) by dialyzed against 0.1 M triethylammonium hydrogen chloride (Stevenson & Furneaux, 1991). Methylation of polysaccharides was performed by the Ciucanu's method (Ciucanu & Kerek, 1984). In the case of TEA-ST-F, it was methylated twice to achieve complete methylation. The methylated polysaccharides were hydrolyzed with 2 M TFA at 120 °C for 1 h, reduced with $NaBD_4$, and acetylated. The partially methylated alditol acetates were analyzed GC using a SP-2330 fused silica capillary column and GC–MS using a DB-5MS fused silica capillary column. Identification of partially methylated alditol acetates was carried out on the basis of relative retention time to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol and its mass fragmentation patterns (Carpita & Shea, 1988). Peak area was corrected using published molar response factors (Sweet, Shapiro, & Albersheim, 1975).

2.6. Spectroscopic analyses of polysaccharides

IR spectra of polysaccharides were recorded with a FT/IR-460plus spectrophotometer using KBr method (Jasco, Tokyo, Japan). NMR spectra were recorded at 303 K on a Varian Unity 500 *plus* spectrophotometer, and sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) was used as an internal reference.

2.7. Cells and viruses

Vero and MDCK cells were grown in Eagle's minimum essential medium (MEM) supplemented with 5% fetal bovine serum (FBS) and kanamycin (60 mg/l). RAW 264.7 cells were grown in Dulbecco's modified MEM (DMEM) supplemented with 10% FBS. HSV-2 (UW264 strain) and influenza A virus (IFV-A, A/NWS/33 strain, H1N1 subtype) were propagated on Vero and MDCK cells, respectively. Those viruses were stored at −80 °C until use. An aliquot of the virus stock was titered by plaque assay.

2.8. Antiviral activity and cytotoxycity of polysaccharides

Vero and MDCK cell monolayers were infected with HSV-2 or influenza virus, respectively, at 0.1 plaque forming unit (PFU) per cell at room temperature. After 1 h of viral infection, the monolayers were washed three times with phosphate-buffered saline (PBS) and incubated in a maintenance medium (MEM plus 2% FBS) at 37 °C. Samples were added either during infection and throughout the subsequent incubation (A) or immediately after the viral

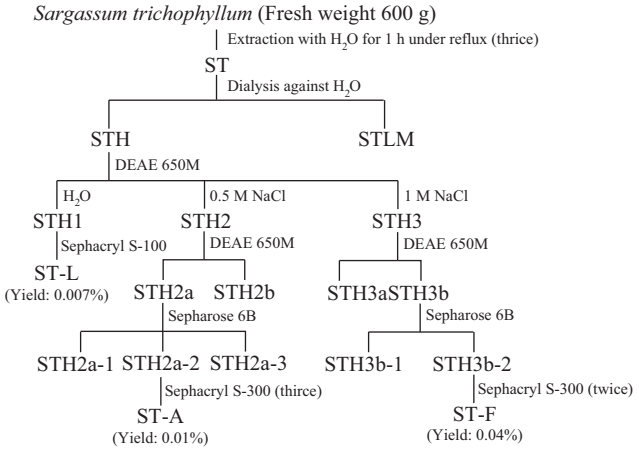

Fig. 1. Scheme for the extraction and purification of polysaccharides from the brown alga *Sargassum trichophyllum*.

infection (B). Virus yields were determined by plaque assay at 1-day incubation point. The 50% inhibitory concentration (IC_{50}) was obtained from the concentration–response curves. For cell growth inhibition study, Vero or MDCK cells were incubated at an initial density of 1×10^4 cells/well in 96-well plates. After cells had been incubated for 1 day at 37 °C, sample was added and the incubation was continued for 3 days. Viable cell yield was determined by the trypan blue exclusion test. The 50% cytotoxic concentration (CC_{50}) was obtained from concentration–response curves. All date were expressed as mean ± SD from triplicate assays. To determine the effect of polysaccharides on direct inactivation of virus particles, HSV-2 or IFV-A (2×10^4 PFU/100 μl) was treated with an equal volume of polysaccharides at 37 °C. After 1.5 h, 100-fold dilutions of the mixture were added to Vero or MDCK cell monolayers for 1 h at room temperature. The cell monolayers were overlaid with media containing 0.8% methylcellulose and 2% FBS to be plaque-assayed.

3. Results

3.1. Isolation of polysaccharides from S. trichophyllum

Extraction and isolation of polysaccharides from *S. trichophyllum* were performed according to Fig. 1. Hot water extract (ST) was fractionated by dialysis to remove the relatively low molecular weight portion (STLM). Crude polysaccharide (STH) was fractionated by anion exchange chromatography into three sub-fractions (STH1, STH2 and STH3). STH1 was suggested to be neutral polysaccharides because it was not bound to the anion exchange resin. STH1 was purified by gel filtration on Sephacryl S-100 HR to yield a colorless polysaccharide (ST-L). STH2 was further purified by anion exchange and gel filtration column chromatographies including DEAE 650M, Sepharose 6B and Sephacryl S-300 HR. Thus obtained purified polysaccharide was named ST-A. STH3 was also purified by the combination of anion exchange and gel filtration to give a colorless polysaccharide (ST-F).

3.2. Characterization of ST-L

Molecular weight of ST-L was estimated to be 6.14×10^3, and its polydispersity index (Mw/Mn = 1.17) revealed that ST-L was isolated as a homogeneous polysaccharide. FT-IR spectrum of ST-L showed no characteristic absorption bands such as >S=O or –COO stretching. Sugar composition analysis revealed that ST-L consisted of glucose with trace amounts of xylose. Methylation analysis indicated that ST-L was composed of non-reducing ter-

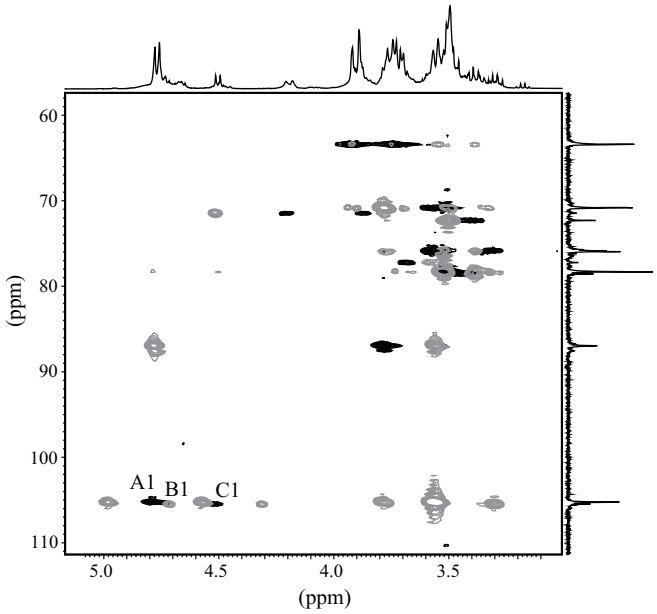

Fig. 2. HSQC and HMBC spectrum of the glucan (ST-L) in D_2O at 300 K.

minal glucopyranose (14.5%), 1,3-linked glucopyranose (69.4%), 1,6-linked glucopyranose (3.4%), and 1,3,6-linked glucopyranose (12.8%). Therefore, it was suggested that ST-L might be a highly branched glucan. We employed NMR analyses to clarify the chemical structure of ST-L. As shown in Fig. 2, three anomeric signals, δ_H 4.78 (*d*, 8.4 Hz) and δ_C 105.2 ppm, δ_H 4.76 (*d*, ~8 Hz) and δ_C 105.3 ppm and δ_H 4.52 (*d*, 8.1 Hz) and δ_C 105.5 ppm were observed in the in HSQC spectrum, and they were named as residue A, B and C, respectively. Therefore, three spin systems were suggested to be present mainly in ST-L. All signals were assigned by the combination of several 2D NMR spectra as shown in Table 1. Residues A, B and C were found to be β-glucose residues since their chemical shift values and coupling constants. Residues A and B were suggested to be 3-substituted and 3,6-disubstituted residues because ^{13}C chemical shift values of the positions were obviously down-field shifted. In the HMBC spectrum, a long range correlation between H-1 of A and C-3 of A and B were observed. Therefore, it was suggested that A residue was linked at 3 positions of A or B residues. On the other hand, H-1 of C residue showed a strong long range correlation to C-6 of B residue. This observation revealed that C residue was connected to 6 position of B residue. These assignments agreed well with the data of methylation analysis, and ST-F was shown to be a branched laminaran.

3.3. Characterization of ST-A

ST-A was obtained as an acidic polysaccharide because it was retained by an anionic exchange column chromatography. Its molecular weight and polydispersity index were estimated to be 2.36×10^4 and 1.15, respectively. FT-IR spectrum showed the absorption bands at 3400 cm^{-1} (OH stretching), 1610 and 1402 cm^{-1} (COO stretching), which were characteristic bands of alginate. In addition, two bands at 1100 and 1036 cm^{-1} responsible for mannuronic and guluronic units, respectively, were also observed. In the ^{13}C NMR spectrum of ST-A (Fig. 3), four anomeric carbon signals at δ 104.0, 103.5, 102.8 and 102.3 ppm were observed and assigned to be C-1 of MG, GG, MM and GM, respectively (Kawarada, Hirai, Odani, Iida, & Nakajima, 1990). Other ring carbon signals were also identical to the reported spectra (Kawarada et al., 1990), therefore, ST-A was identified to be an alginate. In order to

Table 1
^{1}H and ^{13}C NMR spectral data of the glucan (ST-L) in D_2O.

Residue	1	2	3	4	5	6	
A:	105.2	75.9	87.0	70.8	78.3	63.4	
→3)-Glc-(1→	4.78	3.57	3.78	3.51	3.51	3.92	3.73
B:	105.3	75.4	87.5	70.9	77.2	71.4	
→6,3)-Glc-(1→	4.76	3.58	3.78	3.59	3.69	4.21	3.87
C:	105.5	75.9	78.3	72.3	78.5	63.4	
Glc-(1→	4.52	3.30	3.49	3.38	3.44	3.92	3.73

evaluate the content of M and G units, we have investigated the ^{1}H NMR spectrum of ST-A by the procedures as described previously (Chattopadhyay et al., 2010; Torres et al., 2007), and the M/G ratio was calculated to be 1.88.

3.4. Characterization of ST-F

ST-F was isolated as a colorless polysaccharide, and its molecular weight was estimated to be 1.98×10^4 (Mw/Mn = 1.36). Cellulose acetate membrane electrophoresis also revealed that ST-F was an anionic polysaccharide because single band was detected by toluidine blue staining (data not shown). FT-IR spectrum showed an absorption band at 1256 cm^{-1} related to S=O stretching vibration of the sulfate group. The sulfate content was estimated to be 23.5% by barium-rhodizonate method. Colorimetric assays also indicated the presence of small amounts of uronic acids (1.3%). Monosaccharide composition analysis revealed that ST-F consisted of fucose (79.8%) and galactose (20.2%). In order to elucidate the sugar linkages and substitution sites of sulfate groups, methylation analyses were performed against ST-F and its desulfated material (ST-F-D). As shown in Table 2, ST-F-D was mainly composed of non-reducing terminal, 4-, 3-, and 3,4-disubstituted fucose residues. In addition, non-reducing terminal, 2-, and 6-substituted galactose residues were also detected. On the other hand, native polysaccharide (ST-F) showed the presence of 2,3-disubstituted and 2,3,4-trisubstitued fucose residues. By comparison of these data, sulfate groups were suggested to be linked C-2 or C-4 positions of fucose residues. The ^{1}H NMR spectrum of ST-F-D also suggested that this polymer had a complicated structure because many anomeric proton and methyl proton signals were observed in the region between 5.0 and 5.4 ppm and between 1.2 and 1.5 ppm, respectively (data not shown). In addition, those anomeric resonances indicated that the fucose residues in ST-F were suggested to be α-linked residues.

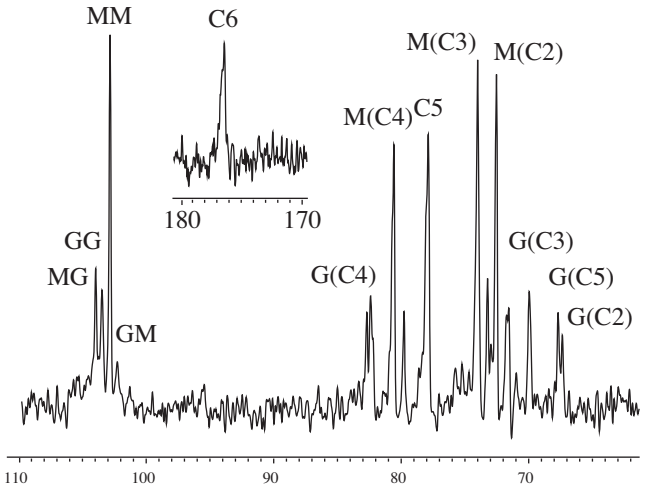

Fig. 3. ^{13}C NMR spectrum of the alginate (ST-A) in D_2O at 303 K at 125 MHz.

3.5. Antiviral activities of polysaccharides

Among the isolated polysaccharides from *S. trichophyllum*, ST-F only showed antiviral activities against HSV-2. ST-F showed a low toxicity on the host cell growth, with the 50% cytotoxic concentration (CC_{50}) being higher than 5000 μg/ml. When it was added to the medium during infection and throughout the incubation (experiment A) or immediately after viral infection (experiment B), the 50% inhibitory concentrations against virus replication (IC_{50}) were 18 and 410 μg/ml, respectively. The resultant selectivity indices (CC_{50}/IC_{50}) of ST-F were >280 and >12 for experiment A and B, respectively. In general, a sample was regarded as possessing antiviral activity when its selectivity index (SI, CC_{50}/IC_{50}) was higher than 10. Therefore, ST-F possesses anti-HSV-2 activity *in vitro*. In addition, the main antiviral target of ST-F might be virus adsorption and/or penetration step(s) on host cell surface because the SI value in the experiment A was higher than that in the experiment B. So far, virucidal effect has been thought to be favorable in preventing from virus infection (Carlucci, Scolaro, Noseda, Cerezo, & Damonte, 2004; Ohta et al., 2009). However, ST-F had no virucidal effects when we examined whether ST-F possessed this effect or not (data not shown).

4. Discussion

As described above, we isolated three polysaccharides, ST-L, -A and -F, from *S. trichophyllum*. It has been reported that *Turbinaria conoides*, which is a related species of *Sargassum* sp., contains laminaran, alginate and fucoidan (Chattopadhyay et al., 2010). In addition, Sinha, Astani, Ghosh, Schnitzler, and Ray (2010) has reported the isolation of alginate and fucoidan from *S. tenerrimum*. Although there are slight differences in chemical characteristics of polysaccharides between *S. trichophyllum* and the others, we could obtain almost the same results as those described in the previous reports. We could isolated a branched β-1,3-D-glucan from *S. trichophyllum*. *Laminaria gurjanovae* has been reported to contain two

Table 2
Results of methylation analyses of fucoidan (ST-F) and desulfated fucoidan.

Methylated sugar	Deduced linkage	Fucoidan	Desulfated fucoidan
2,3,4-Me_3-Fuc	Fuc-(1→	8.9	16.3
2,3-Me_2-Fuc	→4)-Fuc-(1→	16.9	15.3
2,4-Me_2-Fuc	→3)-Fuc-(1→	12.9	34.6
3,4-Me_2-Fuc	→2)-Fuc-(1→	6.9	4.0
2-Me-Fuc	→4,3)-Fuc-(1→	17.3	8.6
3-Me-Fuc	→3,2)-Fuc-(1→	20.8	n.d.
Unmethylated Fuc	→4,3,2)-Fuc-(1→	10.0	n.d.
2,3,4,6-Me_4-Gal	Gal-(1→	1.4	7.3
2,4,6-Me_3-Gal	→4)-Gal-(1→	1.3	n.d.
2,4,6-Me_3-Gal	→3)-Gal-(1→	0.5	1.5
3,4,6-Me_3-Gal	→2)-Gal-(1→	1.2	5.1
2,3,4-Me_3-Gal	→6)-Gal-(1→	n.d.	6.2
3,4-Me_2-Gal	→6,2)-Gal-(1→	0.9	1.3
2,6-Me_2-Gal	→4,3)-Gal-(1→	1.6	2.0
3.6-Me_2-Gal	→4,2)-Gal-(1→	1.0	n.d.

types of laminaran, β-1,3- and β-1,3:1,6-D-glucans (Shevchenko et al., 2007). On the other hand, ST-A was suggested to be an alginate and its M/G ratio was 1.88. Alginate is one of the extensively studied polysaccharides, and there are several reports comparing their chemical characteristics (Davis et al., 2003; Llans, Sauriol, Morin, & Perlin, 1997; Zubia, Payri, & Deslandes, 2008). Based on its M/G ratio, ST-A could be regard as a high mannuronic acid-containing alginate. Finally, ST-F was found to be a fucoidan consisted of fucose and galactose. So far, numerous fucoidans have been obtained from brown algae and their structures seem to be dependent on their origin. Among *Sargassum* sp., a fucan sulfate from *S. horneri* was solely composed of fucose (Hoshino et al., 1998; Preeprame et al., 2001), whereas the fucoidan from *S. stenophyllum* consisted of fucose and galactose (Duarte, Cardoso, Noseda, & Cerezo, 2001). This research also showed that ST-F consisted of fucose and galactose and the linkage modes of those were almost identical with those of fucoidan from *S. stenophyllum*.

When three isolated polysaccharides were applied to anti-HSV-2 tests *in vitro*, ST-F was confirmed to be an antiviral polysaccharide. Its antiviral target(s) were suggested to be virus–host cell interaction, such as virus binding to and penetration onto host cells. Recently, attention has been paid to the management of HSV-2 infection because this virus is recognized as one of important risk factors of HIV spread, and co-infection of HSV-2 with HIV leading to changes in HIV tropism (Pelú, Benetti, & Calistri, 2001). Therefore, ST-F might be a candidate for anti-HSV-2 agent since it showed a potent antiviral activity. Although ST-A showed no anti-HSV-2 activity, Sinha et al. (2010) reported that a sodium alginate (polyguluronic acid-enriched type) possessed anti-HSV-1 effect (SI > 67). When compared with molecular weights between ST-A and the sodium alginate from *S. tenerrimum*, there were no significant differences (23.6 kDa *versus* 26 kDa, respectively). Thus, antiviral potency of alginates might be dependent on their chemical characteristics, *i.e.* M/G ratio, and/or their structures.

References

Berteau, O., & Mulloy, B. (2003). Sulfated fucans, fresh perspectives: Structures, functions, and biological properties of sulfated fucans and an overview of enzymes active toward this class of polysaccharide. *Glycobiology*, *13*, 29R–40R.

Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical Biochemistry*, *54*, 484–489.

Carlucci, M. J., Scolaro, L. A., Noseda, M. D., Cerezo, A. S., & Damonte, E. B. (2004). Protective effect of a natural carrageenan on genital herpes simplex virus infection in mice. *Antiviral Research*, *64*, 137–141.

Carpita, N. C., & Shea, E. M. (1988). Linkage structure of carbohydrates by gas chromatography–mass spectrometry (GC–MS) of partially methylated alditol acetates. In C. J. Biermann, & G. D. McGinnis (Eds.), *Analysis of carbohydrate by GLC and MS* (pp. 157–216). Boca Raton, FL: CRC Press.

Chattopadhyay, N., Ghosh, T., Sinha, S., Chattopadhyay, K., Karmakar, P., & Ray, B. (2010). Polysaccharides from *Turbinaria conoides*: Structural features and antioxidant capacity. *Food Chemistry*, *118*, 823–829.

Chizhov, A. O., Dell, A., Morris, H. A., Reason, A. J., Haslam, S. M., McDowell, R. A., et al. (1998). Structural analysis of laminarans by MALDI and FAB mass spectrometry. *Carbohydrate Research*, *310*, 203–210.

Ciucanu, I., & Kerek, F. (1984). A simple and rapid method for the permethylation of carbohydrates. *Carbohydrate Research*, *131*, 209–217.

Davis, T. A., Llanes, F., Volesky, B., Diaz-Pulido, G., McCook, L., & Mucci, A. (2003). ^{1}H-NMR study of Na alginates extracted from *Sargassum* spp. in relation to metal biosorption. *Applied Biochemistry and Biotechnology*, *110*, 75–90.

Duarte, M. E. R., Cardoso, M. A., Noseda, M. D., & Cerezo, A. S. (2001). Structural studies on fucoidans from the brown seaweed *Sargassum stenophyllum*. *Carbohydrate Research*, *333*, 281–293.

Dubois, M., Gilles, K. A., Hamilton, J. K., Revers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350–356.

Ghosh, T., Chattopadhyay, K., Marschall, M., Karmakar, P., Mandal, P., & Ray, B. (2009). Focus on antivirally active sulfated polysaccharides: From structure–activity analysis to clinical evaluation. *Glycobiology*, *19*, 2–15.

Hayakawa, Y., Hayashi, T., Lee, J.-B., Srisomporn, P., Maeda, M., Ozawa, T., et al. (2000). Inhibition of thrombin by sulfated polysaccharides isolated from green algae. *Biochimica et Biophysica Acta*, *1543*, 86–94.

Hoshino, T., Hayashi, T., Hayashi, K., Hamada, J., Lee, J.-B., & Sankawa, U. (1998). An antivirally active sulfated polysaccharide from *Sargassum horneri* (Turner) C. Agardh. *Biological & Pharmaceutical Bulletin*, *21*, 730–734.

Kawarada, H., Hirai, A., Odani, H., Iida, T., & Nakajima, A. (1990). Structure characterization of alginate and conformational behaviors of various alkali-metal alginates in solution. *Polymer Bulletin*, *24*, 551–557.

Kusaykin, M., Bakunina, I., Sova, V., Ermakova, S., Kuznetsova, T., Besednova, N., et al. (2008). Structure, biological activity, and enzymatic transformation of fucoidans from the brown seaweeds. *Biotechnology Journal*, *3*, 904–915.

Lee, J.-B., Hayashi, K., Maeda, M., & Hayashi, T. (2004). Antiherpetic activities of sulfated polysaccharides from green algae. *Planta Medica*, *70*, 813–817.

Llans, F., Sauriol, F., Morin, F. G., & Perlin, A. S. (1997). An examination of sodium alginate from *Sargassum* by NMR spectroscopy. *Canadian Journal of Chemistry*, *75*, 585–590.

Nagasawa, K., Inoue, Y., & Tokuyasu, T. (1979). An improved method for the preparation of chondroitin by solvolytic desulfation of chondroitin sulfates. *Journal of Biochemistry*, *86*, 1323–1329.

Ohta, Y., Lee, J.-B., Hayashi, K., & Hayashi, T. (2009). Isolation of sulfated galactan from Codium fragile and its antiviral effect. *Biological & Pharmaceutical Bulletin*, *32*, 892–898.

Palú, G., Benetti, L., & Calistri, A. (2001). Molecular basis of the interactions between herpes simplex viruses and HIV-1. *Herpes*, *8*, 50–55.

Preeprame, S., Hayashi, K., Lee, J.-B., Sankawa, U., & Hayashi, T. (2001). A novel antivirally active fucan sulfate derived from an edible brown alga, *Sargassum horneri*. *Chemical & Pharmaceutical Bulletin*, *49*, 484–485.

Shevchenko, N. M., Annastyuk, S. D., Gerasimenko, N. I., Dmitrenok, P. S., Isakov, V. V., & Zvyagintseva, T. N. (2007). Polysaccharide and lipid composition of the brown seaweed *Laminaria gurjanovae*. *Russian Journal of Bioorganic Chemistry*, *33*, 96–107.

Silvestri, L. J., Hurst, R. E., Simpson, L., & Settine, J. M. (1982). Analysis of sulfate in complex carbohydrates. *Analytical Biochemistry*, *123*, 303–309.

Sinha, S., Astani, A., Ghosh, T., Schnitzler, P., & Ray, B. (2010). Polysaccharides from *Sargassum tenerrimum*: Structural features, chemical modification and antiviral activity. *Phytochemistry*, *71*, 235–242.

Stevenson, T. T., & Furneaux, R. H. (1991). Chemical methods for the analysis of sulphated galactans from red algae. *Carbohydrate Research*, *210*, 277–298.

Sweet, D. P., Shapiro, R. H., & Albersheim, P. (1975). Quantitative analysis by various g.l.c. response-factor theories for partially methylated and partially ethylated alditol acetates. *Carbohydrate Research*, *40*, 217–225.

Torres, M. R., Sousa, A. P. A., Silva Filho, E. A., Melo, D. F., Feitosa, J. P. A., de Paula, R. C. M., et al. (2007). Extraction and physicochemical characterization of *Saragassum vulgare* alginate from Brazil. *Carbohydrate Research*, *342*, 2067–2074.

Usov, A. I., & Chizhov, A. O. (1993). New data on the structure of laminaran from *Chondra filum* (L.) Lam. and reserve glucans from other brown algae. *Russian Chemical Bulletin*, *42*, 1597–1601.

Zubia, M., Payri, C., & Deslandes, E. (2008). Alginate, mannitol, phenolic compounds and biological activities of two range-extending brown algae, *Sargassum mangarevense* and *Turbinaria ornata* (Phaeophyta: Fucales), from Tahiti (French Polynesia). *Journal of Applied Phycology*, *20*, 1033–1043.