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Structural analysis and antiviral activity of a sulfated galactan from the red seaweed *Schizymenia binderi* (Gigartinales, Rhodophyta)

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Abstract—Aqueous extraction of gametophytic *Schizymenia binderi* afforded a polysaccharide composed of galactose and sulfate groups in a molar ratio of 1.0:0.89 together with uronic acids (6.8 wt %) and minor amounts of other neutral sugars. Alkali-treatment of the polysaccharide afforded a polysaccharide devoid of 3,6-anhydrogalactose. ^{13}C NMR spectroscopy of the desulfated alkali-treated polysaccharide showed a backbone structure of alternating 3-linked β-D-galactopyranosyl and 4-linked α-galactopyranosyl units that are predominantly of the D-configuration and partly of the L-configuration. Methylation, ethylation and NMR spectroscopic studies of the alkali-treated polysaccharide indicated that the sulfate groups are located mainly at positions O-2 of 3-linked β-D-galactopyranosyl residue and at position O-3 of 4-linked-α-galactopyranosyl residues, the latter is partially glycosylated at position O-2. The sulfated galactan from *S. binderi* exhibited highly selective antiviral activity against *Herpes simplex* virus types 1 and 2, with selectivity indices (ratio cytotoxicity/antiviral activity) >1000 for all assayed virus strains. This compound was shown to interfere with the initial adsorption of viruses to cells.

Keywords: *Schizymenia binderi*; Rhodophyta; Sulfated galactan; Uronic acids; Natural antiviral

1. Introduction

Soluble polysaccharides from a few species of the genus *Schizymenia* (Gigartinales, Rhodophyta) have been studied. Whyte et al.[1] in a study of red algae from British Columbia, reported that the polysaccharide from *Schizymenia pacifica* was a sulfated D-galactan with a low amount of 3,6-anhydrogalactose. Bourgougnon and co-workers,[2,3] found that the polysaccharide from gametophytic *Schizymenia dubyi* is a sulfated glucuronogalactan. No 3,6-anhydrogalactose was detected and 45% of total galactose was in the L-form. On the other hand, Deslandes et al.[4] reported that the water-soluble polysaccharide from *S. dubyi* was a λ-carrageenan type polysaccharide.

Sulfated polysaccharides from red seaweeds have shown antiviral activities.[5–9] For example, the aqueous extract from *S. pacifica* inhibited in vitro avian retrovirus and mammalian retrovirus reverse transcriptase.[10] In other studies, the sulfated polysaccharide isolated from this algae showed antihuman immunodeficiency virus (HIV) activity in vitro,[11] while the sulfated glucuronogalactan from *S. dubyi* inhibited the in vitro replication of HIV.[12,13] Cáceres et al.[14] reported that the polysaccharides from cystocarpic and tetrasporic *Stenogramme interrupta* showed antiviral effects against different

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strains of herpes simplex virus (HSV). The antiherpetic activity of these polysaccharides was similar to that previously found for the κ/ι- and λ-carrageenans isolated from *Gigartina skottsbergii*.[15] This work presents the results of the structural analysis of the soluble polysaccharide from *Schizymenia binderi* and its antiviral activity.

2. Results and discussion

2.1. Native polysaccharide

Aqueous extraction of gametophytic *S. binderi* J. Ag. afforded 17.6 dry weight percent of a polysaccharide composed mainly of galactose and hemi-ester sulfate in a molar ratio of 1.0:0.89, together with 6.8 wt % of uronic acids. Gel permeation chromatography analysis on Sephadex G-200 suggested that it was homogeneous, and the average molecular weight was estimated at 380,000. This value was in good agreement with that determined spectrophotometrically by the reducing-end method (310,800). Reductive hydrolysis of the polysaccharide, followed by acetylation and subsequent GC–MS analysis of the resulting acetylated alditols, indicated the presence of minor amounts of other neutral sugars (Table 1). The FT-IR spectrum showed a shoulder around 1750 cm^{-1} and strong absorbance at 1261.3 cm^{-1} due to the S=O asymmetric stretching vibration of sulfate groups, and a medium intensity band at 844.5 cm^{-1} assigned to S–O stretching vibration of sulfate groups on C-4 of galactose residues.[16,17] The second-derivative spectrum showed new signals, one at 1741.4 cm^{-1} (assigned to the C=O stretching vibration of a carboxyl acid group) and another, a small signal at 817.8 cm^{-1}, which was at lower wave number than the expected value for the S–O stretching vibration of primary sulfate group (820 cm^{-1}).[18] One more band at 583.8 cm^{-1}, due to O–S–O asymmetric deformation of sulfate groups, was present.[19] Furthermore, the second-derivative FT-IR spectrum showed signals at 1643.8, 1549.5 and 1384.1 cm^{-1} assigned to bands I, II and III of the amide function of proteins.[16] No band due to the presence of 3,6-anhydrogalactosyl residues appeared at 930 cm^{-1}, which is in agreement with the results obtained by reductive hydrolysis analysis of the polysaccharide. Moreover, 3,6-anhydrogalactose was not detected by the colorimetric assay of Yaphe and Arsenault.[20] HPLC analysis of the acidic fraction of the product obtained by total acid hydrolysis of the polysaccharide indicated the presence of glucuronic acid and an unknown uronic acid in a 3:1 ratio. No galacturonic, mannuronic or guluronic acid was detected. The nature of the uronic acids was determined by carboxyl reduction of the native polysaccharide followed by total hydrolysis and GC analysis of the alditol acetates. Co-chromatography with authentic samples of peracetates of D-glucitol and L-iditol confirmed the presence of glucuronic acid and identified iduronic acid as the minor uronic acid in the native polysaccharide.

Bourgougnon et al.[3] reported that the soluble polysaccharide from gametophytic *S. dubyi* contained 33.7% of glucuronic acid. The presence of uronic acids in polysaccharides isolated from Rhodophyta has been reported in a few cases.[21–25] Agarose-carrageenan hybrid polysaccharides from *Lomentaria catenata*, containing glucuronic acid have been characterized. This uronic acid is present as a single unit branch at O-4 of the →3-β-D-galactopyranosyl residues.[26] The xylogalactan from *Palmaria decipiens* contained 4.8% uronic acids, which were identified as galacturonic and glucuronic acid in the ratio 1.5:1.0.[27] It is interesting to note that some calcareous red algae synthesized alginic acid, which is the major polysaccharide of Phaeophyta.[28,29]

The ^{13}C NMR spectrum at 70 °C of the partially hydrolyzed polysaccharide was not well resolved and was very complex. It showed a signal at 173.5 ppm, assigned to the carbonyl carbon of the uronic acids, and two groups of anomeric carbons that were tentatively assigned with the aid of literature data.[30–32] In the β-anomeric region, a major broad signal at 104.8 ppm was assigned to the anomeric carbon of an unsulfated 3-linked β-D-galactopyranosyl residue and/or monosulfated at position O-4, linked to a →4-α-D-galactopyranosyl residue monosulfated at position O-3. The signal at 103.7 ppm was assigned to the anomeric carbon of 3-linked β-D-galactopyranosyl residues linked to a →4-α-L-galactopyranosyl residue. In the α-anomeric region, a signal at 101.1 ppm was attributed to C-1 of unsulfated 4-linked α-L-galactopyranosyl residue and/or sulfated at position O-3. The signal at

Table 1. Chemical composition (wt %) of the polysaccharides from *Schizymenia binderi*

Components	Native (%)	Alkali treated (%)
Total sugars	55.3	58.7
Neutral sugars		
Galactose	43.4	50.9
D-galactose[a]	35.8	43.2
L-galactose[a]	7.6	7.7
Glucose	1.1	Tr
Xylose	1.8	2.1
3-*O*-Methyl-galactose[b]	1.5	1.6
3,6-Anhydro-galactose	Tr	Tr
Uronic acids	6.8	3.0
Proteins	9.4 (8.9)[c]	4.9 (5.8)[c]
Sulfate (as $NaSO_3$)	22.2	26.4
Pyruvic acid	ND	ND

Percentages lower than 1% are given as trace (Tr).
ND = not detected.
[a] By GC analysis of diastereomeric derivatives produced by reductive amination with (*S*)-1-amino-2-propanol.
[b] By GC–MS analysis.
[c] By Hartree–Lowry method.