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Polysaccharides from *Caulerpa racemosa*: Purification and structural features

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Abstract

To study the relationship between the structure and anti-viral activity of polysaccharides present in hot water extracted fraction (CrHWE) of the green seaweed *Caulerpa racemosa* we have purified the crude extract by anion exchange chromatography. A neutral glucan (F1) and an acidic heteroglycan sulfate (F3) containing arabinose, xylose and galactose residues were isolated. This neutral fraction (F1), which on partial hydrolysis produces a series of gluco-oligosaccharides having a degree of polymerization ranging from 3 to 18, is made up of α-(1 → 4)-linked glucose residues. The heteroglycan sulfate, a water-soluble polymer, has a molecular mass of 80 kDa. Glycosidic linkage and ^{13}C NMR analysis of the native (F3) and desulfated (F3D) heteroglycan sulfate suggested that this polysaccharide is branched and contains, *inter alia*, (1 → 3)-linked galactose, terminal- and (1 → 4)-linked xylose, and (1 → 4)- and (1 → 3,4)-linked arabinose residues. Sulfate groups, when present, are located at C-3 of (1 → 4)-linked arabinose and C-6 of (1 → 3)-linked galactose units. Degradation of this polysaccharide with trifluoro acetic acid and structural analysis of the resulting fragments by gas liquid chromatography–mass spectrometry (GLC–MS) and matrix-assisted laser desorption ionization-time of flight-mass spectrometry (MALDI-TOF-MS) indicate the presence of two series of oligosaccharides: (i) the first contains two to nine galactose residues and a sulfate group, and (ii) the other one contains an arabinose or xylose, one to eight galactose residues and one sulfate group.

Keywords: *Caulerpa*; Seaweed; Glucan; Heteroglycan sulfate; Partial hydrolysis; Oligosaccharides; MALDI-TOF-MS; NMR

1. Introduction

Seaweeds have been used widely for centuries as food in Asia (Darcy-Vrillon, 1993; Indergaard & Minsaas, 1991), but in western countries, the uses of this biomass span from food, cosmetic and pharmaceutical industries to microbiology and biotechnology (Lewis, Stanley, & Guist, 1988; Renn, 1997; Skjak-Braek & Martinsen, 1991; Stephen, 1995; Zilinskas & Lundin, 1993). In recent years, screening assays of the antiviral activity of extracts from a number of marine algae have led to the identification of a number of sulfated polysaccharides having potent inhibitory effects against herpes simplex virus (HSV) types 1 and 2, human cytomegalovirus (HCMV), human immunodeficiency virus type-1 (HIV-1), respiratory syncytial virus (RSV) and influenza virus (Adhikari et al., 2006; Duarte et al., 2004; Franz, Pauper, & Alban, 2000; Ghosh et al., 2004; Gunay & Linhardt, 1999; Iqbal, Flick-Smith, & McCauley, 2000; Mazumder et al., 2002; Ponce, Pujol, Damonte, Flores, & Stortz, 2003; Witvrow & De Clercq, 1997). These polysaccharides include fucoidans, sulfated galactans, ulvans, sulfated mannans, sulfated heteroglycans, etc. Thus, the antiviral potential of polysaccharides extracted from seaweed becomes of considerable interest.

In a preceding paper (Ghosh et al., 2004) it was shown that water extracted polysaccharide-containing fraction of the green seaweed *Caulerpa racemosa* possesses *in vitro* anti-herpetic activity. The present study reports

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purification of this bioactive fraction and chemical investigation on the purified polysaccharides. Using partial acid hydrolysis, GLC, GLC–MS, NMR and MALDI-TOF mass spectrometry, we have been able to deduce structural features of a neutral glucan and a sulphated heretoglycan.

2. Experimental

2.1. Polysaccharides

The depigmented algal powder prepared from *Caulerpa racemosa* (4 g) on treatment with water (pH 6.5, 3×200 mL) at 80 °C for 30 min yielded a hot water extracted polymeric fraction designated as CrHWE (Ghosh et al., 2004). The alga was collected from the coast of Gujarat, India in August 1995.

2.2. Anion-exchange chromatographic method

A solution of hot water extracted fraction (CrHWE) in 50 mM NaOAc buffer (pH 5.0) was applied to a DEAE–Sepharose FF column (20×2 cm) equilibrated with the same buffer. The column was washed with same buffer (200 mL: fraction F1) and then eluted successively with 0.2 M (fraction F2) and 1.5 M NaOAc (fraction F3) buffer in a stepwise manner. The flow rate of the column was 0.5 mL min^{-1} and elution with each buffer was carried out up to the absence of a positive reaction for carbohydrates with phenol and sulfuric acid. The collected fractions, which had been analyzed for their total sugar contents were pooled, dialyzed and then lyophilized.

2.3. Size exclusion chromatography

Size exclusion chromatography of the heteroglycan sulfate (F3) on Sephacryl S-300 column (90×2.6 cm) using 500 mM sodium acetate buffer (pH 4.0) as eluant was done as described (Mazumder, Lerouge, Loutelier-Bourhis, Driouich, & Ray, 2005). The column was calibrated with standard dextrans (10–500 kDa; a gift from Dr. Tapani Vuorinen).

2.4. Partial acid hydrolysis

Samples (F1 and F3, 100 mg each) were dissolved in 50 ml water in separate tubes and hydrolysed for 75 min at 100 °C with 100 mM trifluoro acetic acid (TFA). The pH of the reaction mixture obtained from the hydrolysis of F3 fraction was adjusted to 6 with 1 M NaOH and the concentrated hydrolysate was desalted using Sephadex G-10 column (90×2.6 cm). The flow rate of the column was 1 ml/min and fractions (10 mL) with K_{av} values ranging from 0 to 0.7 were collected (pool I). This pool was concentrated, diluted with ethanol up to a final concentration of 80% and centrifuged. The supernatant containing ethanol soluble oligosaccharides was concentrated under a stream of nitrogen and lyophilized (named F3-ose). The hydrolysate originated from F1 fraction was, however, diluted with ethanol (4 vol) and then centrifuged. The ethanol soluble oligosaccharide containing sub-fraction (F1-ose) was isolated by lyophilizing the concentrated supernatant.

2.5. Sugar analysis

Total sugars and uronic acids were determined by the phenol–sulfuric acid (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and *m*-hydroxydiphenyl (Ahmed & Labavitch, 1977) assay, respectively. Neutral sugars were released by hydrolysis in 2 M TFA and analyzed as their alditol acetates (Blakeney, Harris, Henry, & Bruce, 1983) by GLC on columns of SGE BP 225 and DB-225 (JW). *myo*-Inositol was used as internal standard, and hydrolytic losses were accounted for by using an external standard. Monosaccharides were identified by thin layer chromatography and gas liquid chromatography–mass spectrometry as described (Ghosh et al., 2005).

2.6. Sulphate estimation and desulfation

Estimation of sulfate by the modified barium chloride method (Craigie, Wen, & van der Meer, 1984) and IR-spectrometry (Rochas, Lahaye, & Yaphe, 1986), and solvolytic desulfation by the method of Nagasawa, Inoue, and Kamara (1977) were carried out as described (Ray & Lahaye, 1995).

2.7. Methylation analysis

Two milligrams of each of the native heteroglycan (F3), its desulfated derivative (F3D) and the glucan (F1) were subjected to two rounds of methylation (Blakeney & Stone, 1985), with the modifications suggested by Stevenson and Furneaux (1991). The permethylated materials were converted into their partially methylated alditol acetates and analysed by GLC and GLC/MS (Shimadzu QP 5050A GLC/MS) as described (Ray, 2006).

2.8. Periodate oxidation

A solution of F1 (10 mg) in 0.25 M formic acid, pH adjusted to 3.7 with 1 M NaOH was treated with of $NaIO_4$ (53.5 mg) as described (Fry, 1988). The oxidized product was hydrolyzed and the liberated monosaccharides were converted into their alditol acetates and analyzed by GLC.

2.9. Spectroscopy

2.9.1. FT-IR

IR spectra (KBr disc) were obtained with a JASCO FT-IR 420 spectrophotometer.

2.9.2. Electron ionization (EI) mass spectrometry

EI mass spectra were recorded with a Shimadzu OP5050A GC–MS instrument at 70 eV.

2.9.3. Matrix assisted laser desorption ionisation-time of flight (MALDI-TOF) mass spectrometry

MALDI-TOF mass spectra of the acid generated oligosaccharides containing fractions (F1-ose and F3-ose) were recorded on a Micromass (Manchester, UK) TOF spec E MALDI-TOF mass spectrometer. Spectra were acquired in the (−) mode for the sulphated oligosaccharide-containing fraction (F3-ose), but in the (+) mode for the neutral oligosaccharides containing fraction (F1-ose). Sample (1 μL) was mixed with 1 μL of the matrix solution (2,5-dihydroxybenzoic acid 10 mg mL^{-1} in TFA:CH_3 CN: 1.75:0.75; v/v) and a total of 1 μL of this solution was applied to a stainless steel sample slide and dried under vacuum.

2.9.4. NMR spectroscopy

The ^{1}H spectrum of F1 fraction was recorded using Bruker DRX-500 NMR spectrometer. Samples were deuterium-exchanged by lyophilization with D_2O and then examined as 0.7% solutions in 99.8% D_2O. The spectrum was recorded at 70 °C with HOD suppression by pre-saturation. The ^{13}C NMR spectrum of the desulfated heteroglycan was recorded on a Bruker-Advance 400 NMR spectrometer provided with a 5-mm probe, at 30 °C.

3. Results and discussion

3.1. Purification of water extracted fraction by anion exchange chromatography

Extraction of the marine green alga *C. racemosa* with hot water yielded a crude polysaccharide-containing fraction (named CrHWE) having activity against Herpes simplex virus (Ghosh et al., 2004). This hot water extracted fraction (CrHWE) showed the presence of 3–4% uronic acid when estimated by *m*-hydroxydiphenyl assay. But no uronic acid was detected by TLC analysis of the acid hydrolysate and IR spectroscopic analysis (no band for carbonyl group was observed in the acid form of the polymers). CrHWE fraction was purified by anion exchange chromatography (Fig. 1) into three sub-fractions (F1, F2 and F3), the yield and the sugar composition of which are given in Table 1. Pool 1 (F1) consisted of unbound polysaccharides and contained glucose as the major sugar. Therefore, this neutral fraction, which consisted of 20% of the total material recovered from the column, contains a glucan. Fraction F2, which was eluted at the beginning of the ionic gradient, was obtained in low amount. This

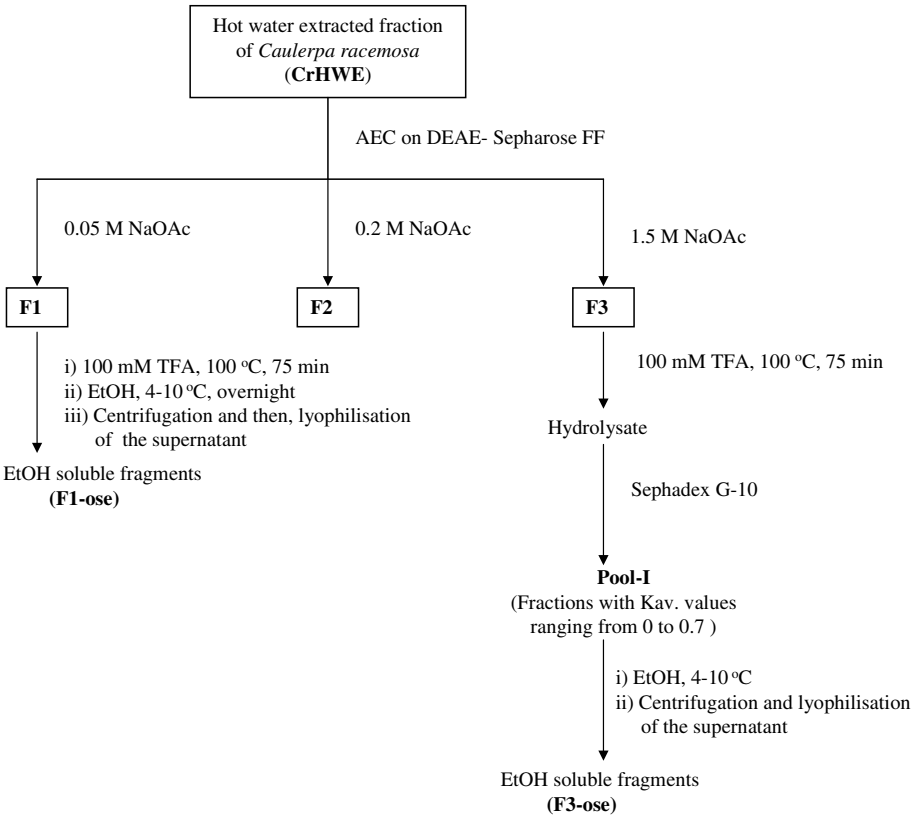

Fig. 1. Scheme for the purification of hot water extracted polysaccharides (CrHWE) of *Caulerpa racemosa* by anion exchange chromatography and isolation of acid generated oligosaccharides.

Table 1
Yields, sulfate content and sugar composition of sub-fractions recovered from anion exchange chromatography of hot water extracted fraction (CrHWE) of *Caulerpa racemosa*, and of other fractions (see text for identification of fractions)

	F1	F2	F3	F3-D	F1-ose	F3-ose
Yield %[a]	20	11	69			
Sulfate[b]	tr	3	12	tr	nd	nd
Ara[c]	2	18	28	30	1	7
Xyl[c]	2	21	27	25	1	1
Man[c]	–	15	tr	–	–	–
Gal[c]	4	35	43	42	2	92
Glc[c]	92	11	2	3	95	tr

–, not detected; nd, not determined; and tr, trace.
[a] Weight percentage of total material recovered.
[b] Percent weight of fraction dry weight.
[c] Percentage mol.

pool (F2) is made up of polymer(s) containing galactose, arabinose, xylose, mannose and glucose residues. Notably, the amount of sulfate group increased with the increase in the ionic strength of the eluant (Table 1). The major fraction F3, which alone accounted for 69% of the total polymers recovered from the anion exchanger, contained higher amount of sulfate (12%; w/w). This fraction that is soluble in water has positive specific rotation $[\alpha]_D^{35} + 39^\circ$(c 0.3, H_2O). The sugar composition of this fraction (Table 1) is similar to the sulfated heteroglycan from *Caulerpa filiformis* (Mackie & Percival, 1961), except that the arabinose content of F3 fraction is significantly higher and the sugar content is much lower than that of earlier report. But, it is well known that seasonal variations and the changes in pH, temperature, etc. of places of from which the algae were collected are responsible for the variation of their compositions (Lahaye & Rochas, 1991). Moreover, the experimental conditions used were also different.

3.2. Chemical characterization of the sulfated xyloarabinogalactan

3.2.1. Molecular mass

Size exclusion chromatography of the heteroglycan sulfate (F3) on Sephacryl S-300 suggests that the polymer is homogeneous. Based on calibration with standard dextrans, the apparent molecular weight of the polysaccharide would be 80 kDa.

3.2.2. Desulfation

The purified heteroglycan (F3) upon solvolytic desulfation yielded compound F3-D in 49% yield. This desulfated polymer (F3-D) composed mainly of galactose, arabinose, xylose together with smaller quantities of glucose (Table 1). Notably, the sugar composition of the native (F3) and the desulfated polysaccharide (F3-D) is very similar.

3.2.3. FT-IR

The FT-IR spectrum of the heteroglycan (F3 fraction) showed a band at 1252 cm^{-1} related to >S═O stretching vibration of the sulfate group (Lloyd, Dodgson, Price, & Rose, 1961; Turvey & Williams, 1962). The peak at 820 cm^{-1} indicated that sulfate groups are located on position six of galactose ring (Hirst, Mackie, & Percival, 1965; Mazumder et al., 2002). In the spectrum of desulfated polymer (F3-D) band characteristics of sulfate groups (1252 cm^{-1}) was almost disappeared.

3.2.4. Linkage analysis

Methylation analysis of the sulfated heteroglycan (F3) of *C. racemosa* demonstrated the presence of a variety of methylated derivatives (Table 2). The amount of sulfate

Table 2
Glycosidic linkage analysis of the constituent sugars of the native heteroglycan (F3), its desulfated derivative (F3-D) and the glucan (F1) of *Caulerpa racemosa*

Partially methylated alditol acetates[a]	Deduced units and substitution pattern	Peak area % of		Peak area % of F1
		F3	F3D	
2,3-Me-Ara[a]	→4)-Ara*p* 3 S[b]-(1→ and/or →5)-Ara*f* 3 S-(1→	7	20	–
2-Me-Ara	→3,4)-Ara*p*-(1→ and/or →3,5)-Ara*f*-(1→	19	13	–
Ara	→2,3,4)-Ara*p*-(1→ and/or →2,3,5)-Ara*f*-(1→	7	2	–
2,3,4-Me-Xyl	T[c]	12	14	–
2,3-Me-Xyl	→4)-Xyl*p*-(1→	10	10	–
Xyl	→2,3,4)-Xyl*p*-(1→	2	2	–
2,3,4,6-Me-Gal	T	1	1	–
2,4,6-Me-Gal	→3)-Gal 6 S-(1→	10	23	–
2,4-Me-Gal	→3,6)-Gal-(1→	18	6	–
2-Me-Gal	→3,4,6)-Gal-(1→	7	5	–
Gal	→2,3,4,6)-Gal-(1→	4	2	–
2,3,6-Me-Glc	→4)-Glc-(1→	1	1	98
2,3-Me-Glc	→4,6)-Glc-(1→	1	1	2

–, not detected.
[a] 2,3-Me-Ara = 2,3-di-*O*-methyl-1,4,5-tri-*O*-acetyl arabinitol etc.
[b] S,$SO_3^=$.
[c] T, terminal.

groups in the purified heteroglycan (F3) as calculated from partially methylated alditol acetates was not in good agreement with the proportions of the experimentally determined sulfate. But it is well known that methylation of sulfated polysaccharides does not always yield reliable proportions of methylated alditol acetates (Patankar, Oehninger, Barnett, Williams, & Clark, 1993; Pereira, Mulloy, & Mourao, 1999; Ray, 2006). The results of methylation analysis of the native sulfated heteroglycan (F3) and its desulfated derivative (F3-D) suggest that galactoses are (1 → 3)-, (1 → 3,6)- and (1 → 3,4,6)-linked. Xyloses are mainly terminal and (1 → 4)-linked, whereas arabinoses are (1 → 4)- and/or (1 → 5)-, and (1 → 3,4)- and/or (1 → 3,5)-linked. Thus, this heteroglycan is branched and contained sulfate at C-3 of arabinose and C-6 of galactose residues. Characterization of 2,3,4-tri-*O*-methyl-1,5-di-*O*-acetyl xylitol indicates that the xylose residues are in the pyranose form, but the ring size of the arabinose residues is not clear. In this context it may be mentioned that the presence of (1 → 4)-linked arabino- and xylo-pyranosyl units in *Cladophora rupestris*, a member of the order Cladophorales, has already been established (Bourne, Johnson, & Percival, 1970; Hirst et al., 1965). Assuming that the genetic information for the synthesis of (1 → 4)-linked pentopyranosyl units, already manifest in Cladophorales heteroglycan, has not been lost in this green algae it may be concluded that the arabinose and xylose residues are in pyranose form.

3.2.5. NMR analysis

^{13}C NMR analysis of polysaccharide is an important tool for the determination of the ring size of the constituent sugars (Capek & Kardosova, 1995; Kardosova & Capek, 1994; Navarro, Cerezo, & Stortz, 2002; Penhoat, Gey, Pellierin, & Perez, 1999; Samuelsen et al., 1999; Usov, Bilan, & Shashkov, 1997; Velde, Knutsen, Usov, Rollema, & Cerezo, 2002). In general, anomeric signals of the pentofuranosyl units appear in region ranging from 107 to 110 ppm whereas that of pentopyranosyl around 97–103 ppm. The fact that the signals for anomeric carbon in the ^{13}C NMR spectrum of the desulfated heteroglycan appear in region ranging from 99.6 to 103.3 ppm indicated that the pentose residues are in pyranose form. Therefore, it may be concluded that the arabinoses of this polysaccharide are (1 → 4)- and (1 → 3,4)-linked. Moreover, the chemical shift values of the anomeric signals of this desulfated heteroglycan indicate the presence of both alpha and beta linkages.

3.2.6. Partial acid hydrolysis

Further information on the structure of this sulfated xyloarabinogalactan (F3) was obtained by partially degrading this polymer with trifluro acetic acid and structural analysis of the resulting fragments. TFA generated oligosaccharides were purified by size exclusion chromatography on Sephadex G-10 and graded precipitation with ethanol. The ethanol soluble oligosaccharide-containing sub-fraction has been designated as F3-ose.

3.2.7. Sugar composition of acid derived oligosaccharides

Sugar compositional analysis of F3-ose revealed the presence of galactose residue together with smaller amount of arabinose and xylose (Table 1). The sugar composition of this sub-fraction differs from that of F3 fraction. Glycosidically linked xylose and arabinose residues, which were preferentially hydrolyzed to the corresponding monomeric units during acid treatment, were lost during size exclusion chromatographic purification of the acid generated oligosaccharides on Sephadex G-10 column. It is well known that pentose sugars are easily hydrolyzed than hexoses (Biermann, 1988). Isolation of oligosaccharides containing high amount galactose residues suggests the presence galactan backbone.

3.2.8. MALDI-TOF-MS analysis of oligosaccharides

MALDI-TOF-mass spectrometry, because of its sensitivity and applicability to the analysis of mixtures is a convenient tool for the structural analysis of oligosaccharides (Fukuyama et al., 2002; Harvey, 1999). We have applied this technique for the analysis of the acid generated oligosaccharides (F3-ose). The MALDI-TOF mass spectrum of F3-ose sub-fraction (Fig. 2), which was acquired in (−) ve mode, reveals the presence of at least nineteen oligosaccharides. Table 3 shows the *m*/*z*, relative intensities (RI) and the assignment of the peaks observed in the linear mass spectrum of sulphated oligosaccharides. Only the $(M-Na)^-$ ion peaks were found in the linear mode spectrum of these compounds. Pseudo molecular ions having *m*/*z* at 421, 583, 745, 907, 1069, 1231, 1393 and 1555 suggests the presence of a series of oligosaccharides containing two to nine galactose residues and a sulfate group. The presence of these oligosaccharides indicated the backbone of the polymer is made up of galactose residues. Moreover, appearance of pseudo molecular ion at *m*/*z* 1555 suggests the presence of an oligomeric building block containing nine galactose residues and a sulphate group. Although mass spectrometry cannot distinguish between diastereoisomers, but sugar compositional analysis of F3-ose sub-fraction indicates the presence of galactose residues. This mass spectrum also indicates the presence of a second series of ions having a mass difference of 162 with *m*/*z* values ranging from 391 to 1525 Da. This series is made up of oligosaccharides containing one to eight galactose residues, one pentose (xylose or arabinose) residue and a sulfate group. The presence of galactose and arabinose/xylose residues in the acid generated oligosaccharides suggests that they are integral part of a single polysaccharide. The parent polysaccharide (F3) contains arabinose and xylose residues as constituent sugars, but oligosaccharides containing only pentose residues were not observed. Notably,

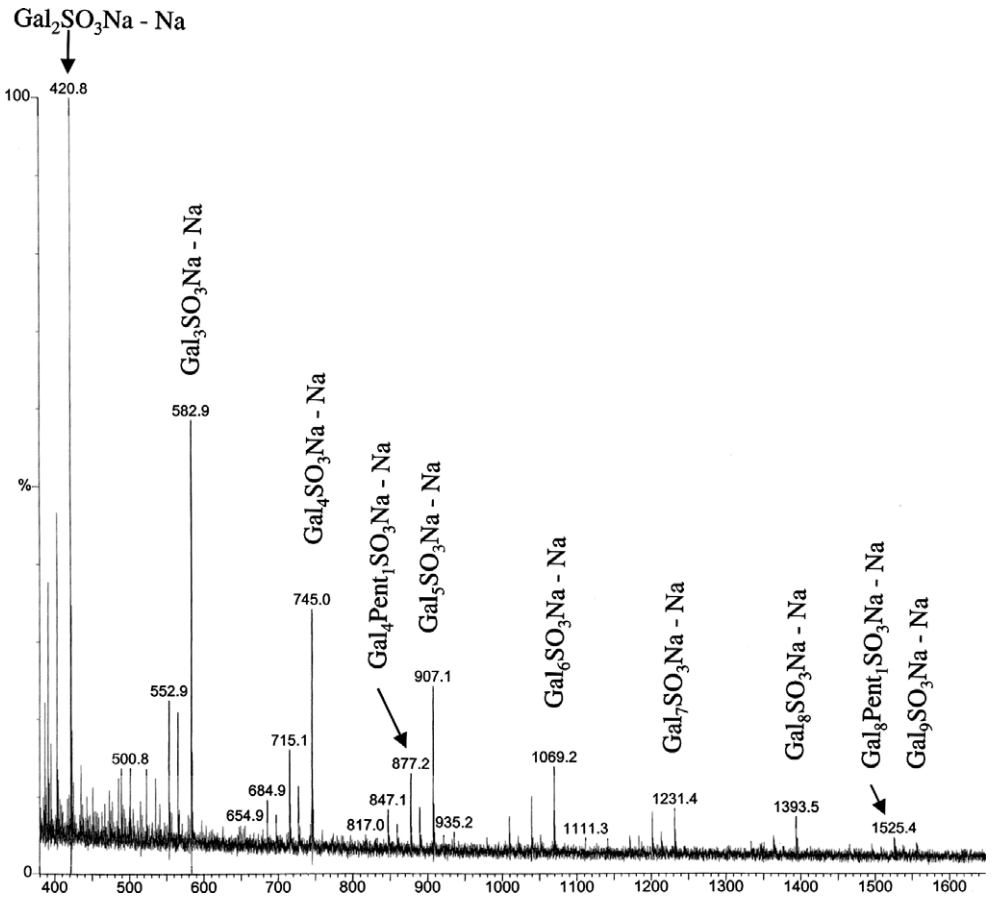

Fig. 2. MALDI-TOF-mass spectrum of oligosaccharides containing sub fraction (F3-ose) generated from the sulphated heteroglycan (F3) of *Caulerpa racemosa* by partial hydrolysis.

Table 3
Negative-ion mode MALDI-TOF-MS of the sulfated oligosaccharides (F3-ose) generated from heteroglycan sulfate (F3) of *Caulerpa racemosa* carried out in the linear mode using 2,4-DHB as matrix

m/z	Relative intensities	Assignment	Compound
421	32	(M−Na)	Hex_2SO_3Na
583	16	(M−Na)	Hex_3SO_3Na
745	10	(M−Na)	Hex_4SO_3Na
907	6	(M−Na)	Hex_5SO_3Na
1069	4	(M−Na)	Hex_6SO_3Na
1231	2	(M−Na)	Hex_7SO_3Na
1393	2	(M−Na)	Hex_8SO_3Na
1555	tr	(M−Na)	Hex_9SO_3Na
391	10	(M−Na)	$Hex_1Pent_1SO_3Na$
553	6	(M−Na)	$Hex_2Pent_1SO_3Na$
715	4	(M−Na)	$Hex_3Pent_1SO_3Na$
877	3	(M−Na)	$Hex_4Pent_1SO_3Na$
1039	2	(M−Na)	$Hex_5Pent_1SO_3Na$
1201	2	(M−Na)	$Hex_6Pent_1SO_3Na$
1363	1	(M−Na)	$Hex_7Pent_1SO_3Na$
1525	1	(M−Na)	$Hex_8Pent_1SO_3Na$
	Total = 100		

tr, trace.

low desorption/ionisation efficiency was observed for these oligosaccharides in the positive-ion mode using DHB as matrix.

3.3. Structural features of the glucan

The neutral glucan containing sub fraction (F1) does not produce blue coloration with iodine. In contrast,

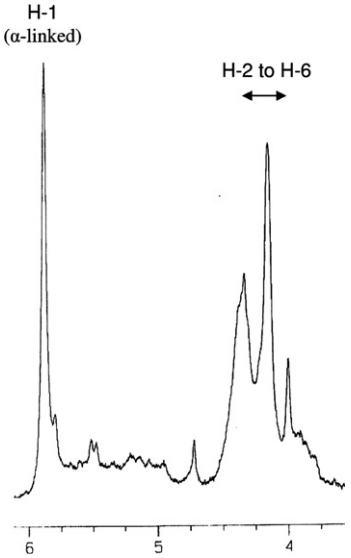

Fig. 3. ^{1}H NMR spectrum of the neutral glucan (F1) isolated by anion exchange chromatography of hot water extracted polymers of *Caulerpa racemosa*.

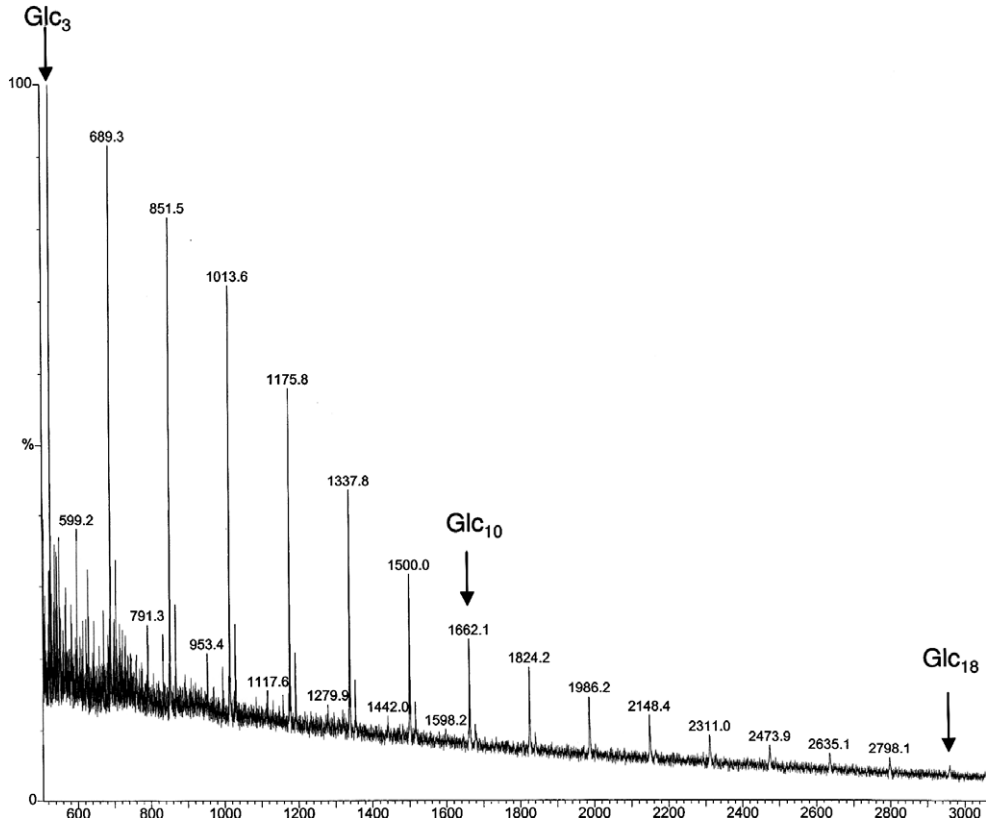

Fig. 4. MALDI-TOF-mass spectrum of oligosaccharides containing sub-fraction (F1-ose) generated from the glucan (F1 fraction) by partial acid hydrolysis. This glucan was isolated as non-retained fraction (F1) during anion exchange chromatography of hot water extracted polymers of *Caulerpa racemosa*.

^{1}H NMR spectrum of this polymer clearly shows the presence of one anomeric signal at 5.827 ppm indicative of α-configuration (Fig. 3). Methylation analysis of this glucan revealed the presence of 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylglucitol (98%) and 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methylglucitol (2%) residues. Periodate oxidation studies of this polymer, which showed that all glucose residues were oxidisable, confirm the results of linkage analysis. Therefore, this glucan has a α-(1 → 4)-linked backbone. Degradation of this glucan with TFA and isolation of the generated fragments by solubilisation in 80% ethanol produces a sub fraction (F1-ose). Sugar compositional analysis indicates that F1-ose is made up of glucose residues (Table 1). The MALDI-TOF mass spectrum of F1-ose fraction, which was acquired in (+) ve mode, indicates the presence of a series of ions having a mass difference of 162 Da with a DP ranging from 3 to as much as 18 (Fig. 4). Therefore, these pseudo molecular ions $(M + Na)^+$, which lack pentosyl units, originated from glucan derived oligosaccharides.

4. Conclusions

In conclusion, the hot water extracted fraction of *C. racemosa*, which showed *in vitro* anti-herpetic activity in earlier study, contains at least two different types of polysaccharides. The polysaccharide, which was not retained in the anion exchange chromatography, is a α-(1 → 4)-linked glucan. The major polymer, a xyloarabinogalactan, is sulfated and had positive specific rotation. The complete structure of this heteroglycan sulfate was not obtained, but some important structural features were established. It was shown that this branched heteroglycan contains two major series of oligomeric building sub-units. The presence of galactan backbone and oligomeric building units containing sulfated galactose residues was indicated. Information regarding the distribution of sulfate groups along the polymer chain and further biological activity study of this purified heteroglycan sulfate is needed. Further work in this regard is in progress.

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