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Sulphated polysaccharides from Indian samples of *Enteromorpha compressa* (Ulvales, Chlorophyta): Isolation and structural features

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Abstract

Heteroglycan and xyloglucan rich fractions were extracted from Indian samples of *Enteromorpha compressa* in 25% yield by sequential extractions with water and alkali. This heteroglycan is sulfated and has an apparent molecular mass of 55 kDa. Chemical structural analysis of this polysaccharide revealed a branched structure having 1,4- and 1,2,4-linked rhamnose 3-sulphate, 1,4-linked glucose, 1,3- and 1,6- linked galactose, 1,4- and terminally linked glucuronic acid and 1,4-linked xylose partially sulfated on *O*-2. Chemical and spectroscopic analysis showed that the 4-M KOH extracted hemicellulosic fraction contained an unusual β-(1,4)-linked linear xyloglucan. Enzyme hydrolysis and analysis of the resulting fragments by matrix-assisted laser desorption ionization-time of flight-mass spectrometry (MALDI-TOF-MS) showed that this linear polymer contained partially sulfated Glc_3Xyl_2 or Glc_4Xyl_2 as oligomeric building subunits.

Keywords: Sulfated heteroglycan; Linear sulfated xyloglucan; *endo*-Glucanase; Oligosaccharides; MALDI-TOF-MS; NMR; *Enteromorpha*; Seaweed

1. Introduction

Polysaccharides produced by seaweeds form the basis of an economically important and expanding global industry (Renn, 1992; Skjak-Braek & Martinsen, 1991; Stephen, 1995). Key products are agars, alginates and carrageenans. The uses of these polymers span from food, cosmetic and pharmaceutical industries to microbiology and biotechnology (Gunay & Linhardt, 1999; Indergaard & Minsaas, 1991; Mazumder, 2006; Skjak-Braek & Martinsen, 1991; Stephen, 1995). Indeed, many consumer products rely on the unique properties of these polysaccharides and would not exist without their availability. Thus, the potential of algal polysaccharides as a raw material in industry becomes of considerable interest.

The marine green algae belonging to Ulvales represent an important biomass that is still little used. Some of them are used as feed or food (Darcy-Vrillon, 1993; Indergaard & Minsaas, 1991) and are involved in algal proliferation in eutrophicated coastal and lagoon waters. One of them, namely *Ulva rigida*, synthesizes a water-soluble polysaccharide referred to as ulvan (Ray & Lahaye, 1995a). This polysaccharide corresponds to water-soluble dietary fibre that resists both human digestive track endogenous enzymes and degradation by colonic bacteria (Andrieux et al., 1998; Bobin-Dubigeon, Lahaye, & Bary, 1997). It forms weak thermo reversible gel in the presence of calcium and boric acid (Lahaye, Ray, Baumberger, Quemener, & Axelos, 1996; Lahaye, Brunel, & Bonnin, 1997) and the gelling mechanism is thought to involve polysaccharide–boric acid complexes and their cross-linking is mediated by calcium. A better description of the chemical structure of cell-wall polysaccharides can lead to a better

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understanding of their different physico-chemical and rheological properties. In continuation to our earlier studies (Lahaye et al., 1996; Lahaye & Ray, 1996; Ray & Lahaye, 1995a; Ray & Lahaye, 1995b; Ray, 2006) on Ulvales cell-wall polysaccharides we now report the structural features of sulfated polysaccharides present in the cell walls of *Enteromorpha compressa* grown along the Indian coastal line. Moreover, using specific enzyme hydrolysis, GC, GC–MS, MALDI-TOF-MS and NMR spectroscopy, we have been able to deduce structural features of an unusual xyloglucan.

2. Materials and methods

2.1. Plant material and preliminary treatments

Algae were collected from Okha coast, Gujarat in December, 1994 and were tentatively identified as *E. compressa* from the morphology and collection site. The gathered material was cut, washed, dried immediately by forced air circulation at 35–40 °C and grinded in a warring blender. The powdered alga (130 g) was sequentially extracted with petroleum ether and acetone in a Soxhlet apparatus (48 h each) to yield 95 g of depigmented alga (DA).

2.2. Isolation of polysaccharides

2.2.1. Extraction with water

Extractions of DA (6 g) with water (w/v: 1:150) at pH 6 were conducted at 80–85 °C for 1.5 h under constant stirring (twice). Separation of the residue from the extract was performed by filtration through a glass filter (G-3). The residue was briefly washed with additional distilled water and the wash was collected to maximize polysaccharide recovery. The combined extracts and washings were dialyzed, and the concentrated retentate was diluted with 4-volume ethanol. The isolated polymeric material was purified by precipitation with 4-volume ethanol (twice). The final pellet was dissolved in water and lyophilized to yield the water extracted fraction (WE, 1.1 g).

2.2.2. Alkali extraction

Hemicellulosic polysaccharides were extracted from the water insoluble residue using the following extraction conditions: (i) 1 M KOH for 16 h at 4–8 °C followed by 4 h at 30–38 °C (B1) and (ii) 4 M KOH for 4 h at 30–38 °C followed by 16 h at 4–8 °C (B4). All extracts were acidified with acetic acid (pH 6), dialyzed, concentrated and finally lyophilized. The resulting insoluble residue was dialyzed against distilled water and finally lyophilized (INS).

2.3. Enzyme hydrolysis

Fraction B4 (1 mg) was suspended in 1 ml of 75 mM NaOAc (pH 5.5) and the mixture incubated with 10 units of *endo*-glucanase (Megazyme International, Ireland) for 24 h at 30–38 °C with occasional shaking (twice). The glucanase resistant polymer was then precipitated in 80% ethanol (v/v) and removed from the suspension by centrifugation. The ethanol soluble fraction containing xyloglucan derived oligosaccharides was concentrated and finally lyophilised (OF). In a separate experiment the water extracted fraction (WE) was incubated (0.5 mg/ml in a 50 mM NaOAc buffer at 30–38 °C for 36 h) with amyloglucosidase (60 U per 1 mg). One unit, U, is defined as the amount that liberates 1 μmol reducing sugar per min. After incubation, the resulting digest was heated at 100 °C for 15 min to inactivate the enzyme.

2.4. Size exclusion chromatography (SEC)

The WE fraction (150 mg) was chromatographed on a Sephacryl S-300 column (90 cm × 2.6 cm) using 500 mM sodium acetate buffer (pH 5.0) as eluant. The flow rate of the column was 0.3 ml/min and collected fractions were checked for the total sugar and uronic acid by the phenol–sulfuric acid (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and *m*-hydroxydiphenyl (Ahmed & Labavitch, 1977) assay, respectively. The total and void volume of the column determined as the elution volume of potassium hydrogen phthalate and dextran (500 kDa), respectively. The column was calibrated with standard dextrans (500, 70, 40 and 10 kDa).

2.5. Sugar analysis

Total sugars and uronic acids were determined by the phenol–sulphuric acid (Dubois et al., 1956) and *m*-hydroxydiphenyl (Ahmed & Labavitch, 1977) assay, respectively. All fractions were hydrolyzed with 2 M trifluoro acetic acid (2 h, 100 °C) for measurement of individual neutral sugar. For water insoluble residues this hydrolysis was followed by a treatment of the resulting residue with 72% (w/w) H_2SO_4 for 1 h at room temperature and then with 1 M H_2SO_4 for 2 h at 100 °C. Sugars were reduced, acetylated and analyzed as their alditol acetate by GLC (Blakeney, Harris, Henry, & Bruce, 1983) on columns of 3% SP-2340 on Supelcoport 100–120 mesh, and DB-225 (JW) and by GLC/MS (Shimadzu QP 5050A). Myo-inositol was used as internal standard. Sugars in the acid hydrolysate were also analyzed by thin-layer chromatography as described (Mazumder, Morvan, Thakur, & Ray, 2004).

2.6. Amino acid analysis

Protein and amino acids were analyzed as described (Mazumder et al., 2004).

2.7. Sulfate estimation and desulfation

Estimation of sulfate by modified barium chloride method and FT-IR spectroscopy, and solvolytic desulfation were carried out as described (Ghosh et al., 2004).

2.8. Carboxyl reduction

Reduction of uronic acid of the desulfated polymer (WE-D) was carried out by the method of York, Darvill, O'Neill, Stevenson, and Albersheim (1985).

2.9. Methylation analysis

The water extracted fraction (WE), its desulfated (WE-D) and desulfated-carboxyl reduced (WE-DR) derivatives and the desulfated derivative (B4D) of the 4 M KOH extracted fraction (2 mg of each) were subjected to two rounds of methylation (Blakeney & Stone, 1985). The methylated polysaccharides were hydrolyzed, and the liberated glycoses converted into their partially methylated alditol acetates and analysed by GLC and GLC/MS as described previously (Ray & Lahaye, 1995a).

2.10. IR spectroscopy

Recording of IR spectra were carried out as described previously (Ghosh et al., 2004).

2.11. MALDI-TOF-mass spectrometry

MALDI-TOF-MS analysis of the *endo*-glucanase generated aqueous 80% ethanol soluble oligosaccharides (fraction OF) was carried out as described (Ghosh et al., 2005). Mass spectrum was recorded using 2,5-dihydroxybenzoic acid (10 mg/ml) as matrix. Two microliters of the ethanol soluble fraction (OF) was mixed with 2 μl of the matrix solution in TFA:CH_3CN (1.75: 0.75; v/v). A 2 μl portion of this solution was applied on a stainless steel sample plate and allowed to dry under vacuum.

2.12. NMR spectroscopy

The ^1H spectrum of the polysaccharide was recorded using Bruker DRX-500 NMR spectrometer. The xyloglucan rich fraction (B4, 10 mg) was heated (at 70 °C for 30 min) with water (1 ml), centrifuged and the resulting supernatant lyophilized. The freeze-dried sample was deuterium-exchanged by lyophilization with D_2O and then examined in 99.9% D_2O.

3. Results and discussion

3.1. Composition of the cell-wall material and fractions isolated there from

The depigmented algal powder (DA) prepared from marine green alga *E. compressa* grown along the coast of Okha, India contained glucose, xylose, rhamnose and galactose as neutral sugar. Total sugar content of this powder was 36% of which about one sixth were uronic acids (Table 1).

Extraction of DA with different inorganic solvents was carried out as shown (Fig. 1). The water extracted fraction (WE) was amounted to 18% of the depigmented algal powder (DA) dry weight and contained 43% total sugar on the basis of fraction dry weight. Sugar compositional analysis revealed that it consists of polysaccharides containing a high amount of rhamnose together with a smaller amount of glucose, xylose and galactose units (Table 1). Thin layer chromatographic analysis of the monosaccharides present in the hydrolysate indicates the presence of, in addition to neutral sugars, an uronic acid with R_f value similar to that of glucuronic acid. The sugar composition of WE fraction is qualitatively similar to the heteroglycan isolated from other *Ulva* spp. (Dodson & Aroson, 1978; McKinnel & Percival, 1962; Medcalf et al., 1972; Percival & McDowell, 1967; Percival & McDowell, 1967; Ray & Lahaye, 1995a; Ray & Lahaye, 1995b; Ray, 2006), except that no iduronic acid was detected as reported by Quemener, Lahaye, and Bobin-Dubigeon (1997). Moreover, the water extracted fraction of present study contained significantly higher amount of galactose and glucose residues. Notably this fraction does not give colour characteristic of starch when reacted with iodine solution and is not hydrolyzed with amyloglucosidase.

Table 1
Yields and monosaccharide composition of fractions obtained from the marine green alga *Enteromorpha compressa* (see text for identification of fractions)

	DA	WE	WE-D	WE-DR	B1	B4	B4-D	INS
Yield[a]	100	18			4	3		36
Sulfate[b]	nd	6	tr	–	3	3	tr	nd
UA[b]	5	14	25	3	6	4	4	3
NS[b]	31	29	46	61	45	42	52	38
Rha[c]	16 (1)[d]	57	53	48	13	7	6	9
Xyl[c]	18 (3)	15	19	15	32	35	32	22
Gal[c]	6 (tr)	10	8	3	15	8	6	3
Glc[c]	60 (96)	18	20	34	40	50	56	66

nd, not determined; tr, trace; –, not detected; NS, neutral sugar; UA, uronic acid.
[a] Percent weights of depigmented alga dry weight.
[b] Percent weight of the fraction.
[c] Mol% of anhydro sugar.
[d] Values in the parenthesis were obtained after Saeman's hydrolysis.

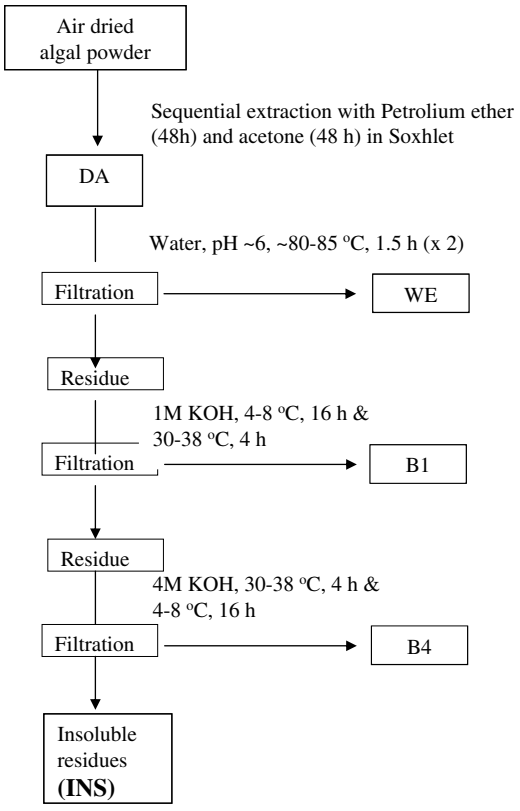

Fig. 1. Scheme for the isolation of polysaccharides from the green seaweed *Enteromorpha compressa*.

As shown, 7% of the DA dry weight was recovered from 1 M- and 4 M KOH extracted fractions (Table 1). Sugar composition analysis shows that xylose and glucose together account for more than 70% of the total carbohydrate of the 1 M- and 4 M KOH extracted fractions (B1 and B4, respectively), which indicates the presence of xyloglucan. Finally, after acid hydrolysis, the alkali insoluble residue (INS) shows very high glucose content (66%) demonstrating the presence of cellulose in this fraction.

3.2. Structural features of the water extracted heteroglycan

The water extracted polymer (WE) contained sulphate groups. Indeed, the IR spectrum of this fraction shows a band at 1254 cm^{-1} related to the >S=O stretching of the sulphate group (Lloyd, Dodgson, Price, & Rose, 1961). Another band at 830 cm^{-1} arising from secondary equatorial sulphate groups of polysaccharides were also detected.

The WE fraction also contained 8% protein and the most abundant amino acids were glutamic acid/glutamine and aspartic acid/asparagine (Table 2). Other abundant amino acids included alanine and glycine.

3.2.1. Molecular mass

The water extracted sulphated heteroglycan was analyzed by size exclusion chromatography (Fig. 2). Based on calibration with standard dextrans, the apparent molecular weight of the polysaccharide would be 55 kDa.

Table 2
Amino acid composition of water extracted fraction (WE) obtained from the green seaweed *Enteromorpha compressa*

Amino acid	Mol%
Aspartic acid/asparagine	17
Threonine	4
Serine	4
Glutamic acid/glutamine	21
Proline	2
Glycine	11
Alanine	11
Valine	6
Isoleucine	2
Leucine	4
Tyrosine	2
Phenylalanine	3
Histidine	1
Lysine	3
Arginine	8

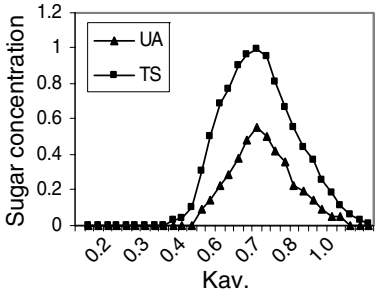

Fig. 2. SEC elution profile of heteroglycan sulfate (WE) obtained from *E. compressa*. Collected fractions were analyzed for total sugar content by phenol–sulfuric acid. Elution of polysaccharide was expressed as a function of the partition coefficient K_{av} [$K_{av} = (V_e - V_o)/(V_t - V_o)$ with V_t and V_o being the total and void volume of the column determined as the elution volume of potassium hydrogen phthalate and dextran (500 kDa), respectively and V_e is the elution volume of the sample]. TS = total sugar. UA = uronic acid.

3.2.2. Desulfation and reduction

The water extracted fraction (WE) upon solvolytic desulphation generates compound WE-D in 40% yield. Notably the sugar composition of this desulphated polymer (WE-D) is similar to that of the native sulphated heteroglycan (Table 1). In the IR spectrum of the desulphated polymer bands characteristics of sulphate groups disappeared.

The desulphated and then reduced polymer (WE-DR) derived from WE fraction showed a marked increase in glucose content confirming the presence of glucuronic acid in the acidic heteroglycan (Table 1).

3.2.3. Linkage analysis

Methylation analysis of the sulphated heteroglycan of *E. compressa* yielded a variety of partially methylated alditol acetates (Table 3). The amount of sulphate groups in the native polysaccharide as calculated from partially methylated alditol acetates was not in good agreement with the experimentally determined sulphate. Although the proportion of methylated derivatives remain unchanged after an

Table 3
Partially methylated alditol acetates derived from sulfated glycuronan (WE) of *E. compressa,* its desulfated (WE-D) and desulfated-reduced (WE-DR) derivatives, and the desulfated derivative (B4-D) of the xyloglucan rich fraction

Monomers[a]	Peak area[b]			
	WE	WE-D	WE-DR	B4-D
2,3,4,6-Glc[a]	–	–	8	–
2,3,6-Glc	13	12	28	60
2,4,6-Gal	1	1	2	1
2,3,4-Gal	2	1	2	1
2,3-Xyl	13	17	13	30
3-Xyl	6	1	1	–
2,3,4-Rha	1	1	1	–
3,4-Rha	3	3	1	1
2,3-Rha	12	53	34	5
2,4-Rha	1	1	–	–
2-Rha	36	1	tr	–
3-Rha	1	8	9	2
Rha	11	1	1	tr

–, not detected; tr, trace.
[a] Linkage of monosaccharides.2,3,4,6-Glc denotes 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol, etc.
[b] Percentage of total area of the identified peaks.

additional methylation cycle (data not shown), it is probable that there was incomplete methylation. In fact, methylation of sulphated polysaccharides does not always yield reliable proportions of methylated alditol acetates (Patankar, Oehninger, Barnett, Williams, & Clark, 1993; Pereira, Mulloy, & Mourao, 1999). The results of methylation analysis of the sulphated heteroglycan (WE) and its derivatives (WE-D and WE-DR) suggest that xyloses are 1,4-linked. Rhamnoses are terminal, 1,4- and 1,2,4-linked. The presence of 1,3- and 1,6-linked galactosyl, and 1,4-linked glucosyl residues was also indicated. This is the first report showing the presence of 1,3- and 1,6-linked galactose residues in the water extracted fraction of Ulvales. Glucuronic acid was 1,4- and terminally linked. Moreover, sulphate groups, when present, reside at *O*-2 of xylose and *O*-3 of rhamnose residues. The partially desulphated and uronic acid reduced water soluble polysaccharides from *Ulva lactuca* were reported (Percival & McDowell, 1967) to contain 1,3,4-, 1,2,3-, 1,4-linked rhamnose, 1,3-, 1,4-linked xylose, 1,3,6-, 1,4- and 1,3-linked glucose (from reduced glucuronic acid) and a structural repeating unit of *O*-L-rhamnopyranosyl-(1 → 4)-*O*-D-xylopyranosyl-(1 → 3)-D-glucopyranose (reduced glucuronic acid). On the basis of methylation, periodate oxidation and IR analyses it was reported that sulphate groups localized on *O*-2 of rhamnose (Percival & McDowell, 1967). However, more recent studies indicated that the sulfated heteroglycan isolated from Ulvales contained, *inter alia*, 1,2,4-linked rhamnose 3-sulphate residues (Ray & Lahaye, 1995a).

3.3. Structural features of the xyloglucan

The result of sugar compositional analysis of the 4 M KOH extracted hemicellulosic fraction (B4) indicates the presence of xyloglucan type polymer (Table 1). The presence of a linear xyloglucan in *U. lactuca* a member of the order Ulvales has already been reported (Lahaye, Jegou, & Buleon, 1994). FT-IR spectrum of B4 fraction reveals the presence of sulphate groups. Solvolytic desulphation of B4 produces compound B4-D in 48% yield. FT-IR spectrum of the desulphated polymer (B4-D) shows no absorption bands characteristics of sulphate groups. The sugar composition of this desulphated polymer (B4-D) is similar to that of the native sulphated xyloglucan (Table 1).

3.3.1. Methylation analysis

Linkage analysis of the desulphated derivative (B4-D) of the 4 M KOH extracted fraction revealed the presence of, *inter alia*, 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylglucitol (60 mol%) and 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methylxylitol (30 mol%) indicating that both glucose and xylose residues are 1,4-linked (Table 3). Therefore, xyloglucan present in the seaweed *E. compressa* is linear. Xyloglucans of higher plants, in contast, are branched polymers and contained a cellulosic (i.e., poly-(1 → 4)-β-D-Glc*p*) backbone, to which α-D-Xyl*p* residues are linked (Fry, 1989).

3.3.2. ^{1}H NMR analysis

The ^{1}H NMR spectrum of B4 fraction (Fig. 3) clearly shows the presence five anomeric doublets between 4.29 and 4.59 ppm. The values of coupling constants of these doublets, which ranged between 6.06 and 8.25 Hz and their chemical shift values, suggest that the glucose and the xylose residues are β-linked. Only one doublet would have been expected from distinct β-glucan and β-xylan chains, which would also been insoluble in water. Therefore, both glucose and xylose occur on the same molecule. In contrast to the xyloglucan of higher plants where the xylose residues are α-linked, the anomeric configuration of the xylose residues of this xyloglucan is β. On the basis of the glycosidic linkage and ^{1}H NMR analysis it may be concluded that the unusual xyloglucan of *E. compressa* is a linear polysaccharide and contained β-(1 → 4)-linked xylopyranosyl and glucopyranosyl residues.

3.3.3. Enzyme hydrolysis

Further information on the structure of polymers present in xyloglucan rich fraction (B4) was obtained by enzymatic degradation of this fraction and characterization of the generated oligosaccharides. Indeed, B4 was treated with an *endo*-(1 → 4)-β-D-glucanase, an enzyme known to cleave β-(1 → 4)-glucosidic linkages of the xyloglucan backbone (Fry, 1989). The enzyme-resistant fraction was removed from the digest by precipitation with 80% (v/v) ethanol. *endo*-Glucanase generated xyloglucan derived oligosaccharides containing fraction, namely OF, was recovered from the supernatant of the digest. Sugar compositional analysis of OF fraction revealed the presence of xylose (32 mol%) and glucose (64 mol%) as major constituent sugars. Thus, both glucose and xylose residues occur on the same polysaccha-

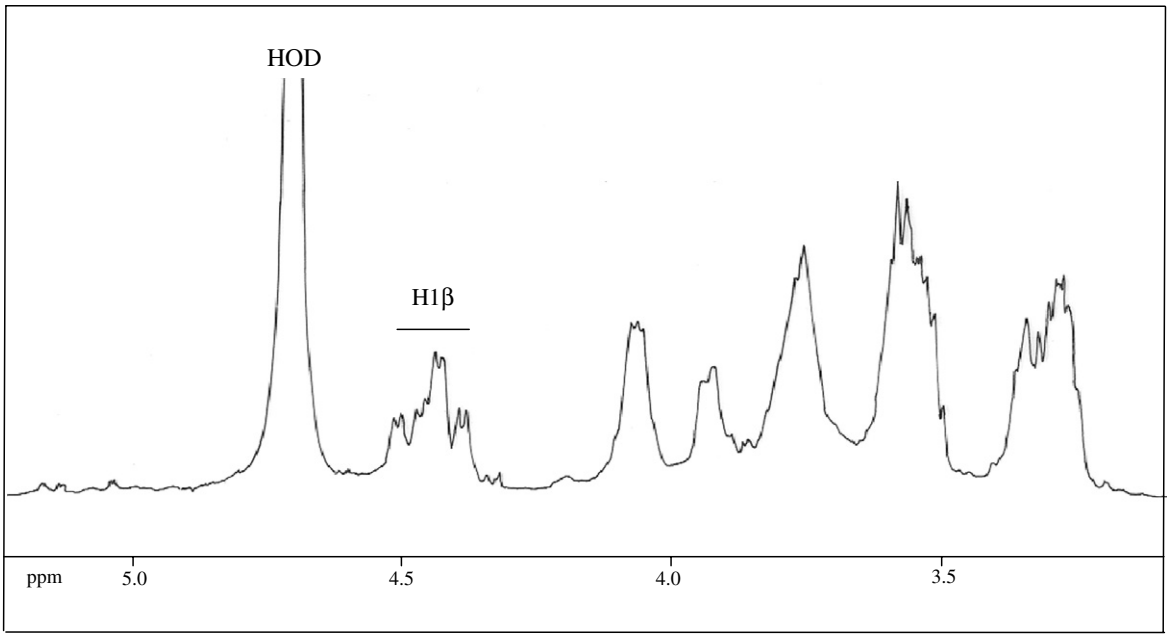

Fig. 3. ^{1}H NMR spectrum at 500 MHz of xyloglucan of *E. compressa*. The spectrum was recorded at 70 °C for sample in D_2O solution. H1β refers to β–anomeric protons of glucose and xylose in xyloglucan polymers, whereas HOD refers to signals from protons of HOD molecules.

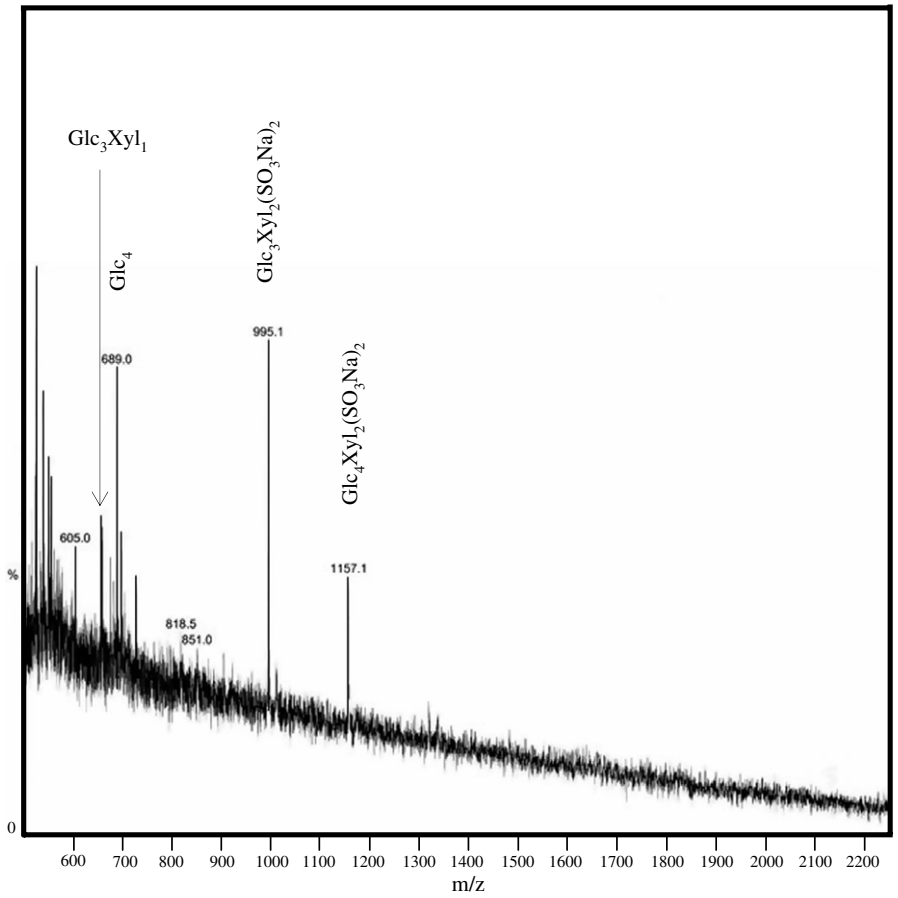

Fig. 4. MALDI-TOF-mass spectrum of oligosaccharides (OF) generated from *E. compressa* xyloglucan by *endo*-glucanase degradation.

ride. Moreover, generation of oligosaccharides by *endo*-glucanase confirms the presence β-(1 → 4)-linked glucose residues in this xyloglucan.

3.3.4. MALDI-TOF-MS

MALDI-TOF-mass spectrometry, because of its sensitivity and applicability to the analysis of mixtures is a con-

venient tool for the structural analysis of oligosaccharides (Harvey, 1999). We have applied this technique for the analysis of enzyme-generated xyloglucan derived fragments (OF). MALDI-TOF mass spectrum of OF fraction shows the presence of four oligosaccharides (Fig. 4). Pseudomolecular ions at m/z 659, 689, 995 and 1157 correspond to Hex_3Pent_1 (Hex = hexose and Pent = pentose residues), Hex_4, $Hex_3Pent_2(SO_3Na)_2$ and $Hex_4Pent_2(SO_3Na)_2$, respectively on the basis of their molecular weight. Taking into consideration the sugar composition data Hex_3 $Pent_1$, Hex_4, $Hex_3Pent_2(SO_3Na)_2$ and $Hex_4Pent_2(SO_3Na)_2$ could be assigned to Glc_3Xyl_1, Glc_4, $Glc_3Xyl_2(SO_3Na)_2$ and $Glc_4Xyl_2(SO_3Na)_2$, respectively. Pseudomolecular ion at m/z 689 corresponding to Glc_4 suggests the presence of an oligomeric building block containing four glucose residues. Thus, *endo*-glucanase is useful in determining the structural features of *E. compressa* xyloglucan.

4. Conclusion

In conclusion, the structural features of the water extracted sulphated heteroglycan of *E. compressa* is close to that of "ulvan" a polysaccharide isolated from other Ulvales, except that it contained significantly higher amount of glucose residues.

The hemicellulosic fraction extracted by 4 M KOH contained a partially sulphated linear β-(1 → 4)-linked xyloglucan. The determination of the structure of this unusual polymer was based on NMR and permethylation data. In addition, MALDI-TOF MS of *endo*-glucanase generated fragments suggested that this xyloglucan is composed of partially sulphated Glc_3Xyl_2 and Glc_4Xyl_2 as building subunits. However, the distribution of sulphate groups along the polymer chain and the sequence of sugar residues are not known. Further work in this regard is in progress in our laboratory.

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References

Ahmed, A., & Labavitch, J. M. (1977). A simplified method for accurate determination of cell wall uronide content. *Journal of Food Biochemistry, 1*, 361–365.

Andrieux, C., Hibert, A., Houari, A. M., Bensaada, M., Popot, F., & Szylit, O. (1998). *Ulva lactuca* is poorly fermented but alters bacterial metabolism in rats inoculated with human faeccal flora from methane and non-methane products. *Journal of the Science of Food and Agriculture, 77*, 25–30.

Blakeney, A. B., Harris, P., Henry, R. J., & Bruce, A. B. (1983). A simple rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydrate Research, 113*, 291–299.

Blakeney, A. B., & Stone, B. A. (1985). Methylation of carbohydrates with lithium methylsulphinyl carbanion. *Carbohydrate Research, 140*, 319–324.

Bobin-Dubigeon, C., Lahaye, M., & Bary, J.-L. (1997). Human colonic bacterial degradability of dietary fibres from sea-lettuce (*Ulva* sp.). *Journal of the Science of Food and Agriculture, 73*, 149–159.

Darcy-Vrillon, B. (1993). Nutritional aspects of the developing use of marine macro algae for the human food industry. *International Journal of Food Science & Nutrition, 44*, S23–S35.

Dodson, J. R., & Aroson, J. M. (1978). Cell wall composition of *Enteromorpha intentinalis*. *Botanica Marina, XXI*, 241–246.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry, 28*(3), 350–366.

Fry, S. C. (1989). The structure and functions of xyloglucan. *Journal of Experimental Botany, 40*, 1–11.

Ghosh, P., Adhikari, U., Ghosal, P., Pujol, C. A., Carlucci, M. J., Damonte, E. B., et al. (2004). *In vitro* anti-herpetic activity of sulfated polysaccharide fractions from *Caulerpa racemosa*. *Phytochemistry, 65*, 3151–3157.

Ghosh, P., Ghosal, P., Thakur, S., Lerouge, P., Loutelier-Bourhis, C., Driouich, A., et al. (2005). Non-starch polysaccharides from *Sesamum indicum*: Isolation and structural features. *Food Chemistry, 90*, 719–726.

Gunay, N. S., & Linhardt, R. J. (1999). Heparinoids: structure, biological activities and therapeutic applications. *Planta Medica, 65*, 301–306.

Harvey, D. J. (1999). Matrix assisted laser desorption/ionization mass spectrometry of carbohydrates. *Mass Spectrometry Reviews, 18*, 349–451.

Indergaard, M., & Minsaas, J. (1991). Animal and human nutrition. In M. D. Guiry & G. Blunden (Eds.), *Seaweed resources in Europe, uses and potential* (pp. 21–64). Chichester: Wiley.

Lahaye, M., Brunel, M., & Bonnin, E. (1997). Fine Chemical structure analysis of oligosaccharides produced by ulvan lyage degradation of the water soluble cell wall polysaccharides from Ulva sp. (Ulvales, Chlorophyta). *Carbohydrate Research, 304*, 325–333.

Lahaye, M., Jegou, D., & Buleon, A. (1994). Chemical characteristics of insoluble glucans from the cell wall of the marine green alga *Ulva lactuca* (L.) Thuret. *Carbohydrate Research, 262*, 115–125.

Lahaye, M., & Ray, B. (1996). Cell-wall polysaccharides from the marine green algae *Ulva rigida* (Ulvales, Chlorophyta)- 3 NMR analysis of ulvan oligosaccharides. *Carbohydrate Research, 283*, 161–173.

Lahaye, M., Ray, B., Baumberger, S., Quemener, B., & Axelos, M. A. V. (1996). Chemical characterisation and gelling properties of cell-wall polysaccharides from species of *Ulva* (Ulvales, Chlorophyta). *Hydrobiologia* (326–327), 473–480.

Lloyd, A. G., Dodgson, K. S., Price, R. B., & Rose, F. A. (1961). I. Polysaccharide Sulphates. *Biochimica Biophysica Acta, 46*, 108–115.

Mazumder, S., Morvan, C., Thakur, S., & Ray, B. (2004). wall polysaccharides from Chalkumra (*Benincasa hispida*) fruit. Part I. Isolation and characterization of pectins. *Journal of Agriculture and Food Chemistry, 52*, 2356–2362.

Mazumder, S. (2006). *Industrial polysaccharides from Benincasa and Gracilaria: structure, physico-chemical properties and physiological activity*. PhD thesis, The University of Burdwan, Burdwan, India.

McKinnel, J. P., & Percival, E. (1962). Structural investigations on the water soluble polysaccharide of the green seaweed *Enteromorpha compressa*. *Journal of Chemical Society*, 3141–3148.

Medcalf, D.G., Root, C.F., Craney, C.L., Mukhopadhyay, D., Miller, C.K., & Hopewell, W.D. (1972). Chemical characterization of mucilaginous polysaccharides from Ulvaceae species native to the Puget Sound. *Proceeding of the international seaweed symposium*, 7 (pp. 541–547).

Percival, E., & McDowell, R. H. (1967). *Chemistry and enzymology of marine algal polysaccharides*. London: Academic Press, p. 219.

Patankar, M. S., Oehninger, S., Barnett, T., Williams, R. L., & Clark, G. F. (1993). A revised structure for fucoidan may explain some of its biological activities. *Journal of Biological Chemistry, 268*, 21770–21776.

Pereira, M. S., Mulloy, B., & Mourao, P. A. S. (1999). Structure and anticoagulant activity of sulfated fucans. *Journal of Biological Chemistry, 274*, 7656–7667.

Quemener, B., Lahaye, M., & Bobin-Dubigeon, C. (1997). Structure determination in ulvans by a chemical-enzymatic method coupled to high performance anion exchange chromatography. *Journal of Applied Phycology, 9*, 179–188.

Ray, B. (2006). from *Enteromorpha compressa*: isolation, purification and structural features. *Carbohydrate Polymers, 66*, 408–416.

Ray, B., & Lahaye, M. (1995a). Cell-wall polysaccharides from the marine green algae *Ulva rigida* (Ulvales, Chlorophyta) – 2. Chemical structure of ulvan. *Carbohydrate Research, 274*, 313–318.

Ray, B., & Lahaye, M. (1995b). Cell-wall polysaccharides from the marine green algae *Ulva rigida* (Ulvales, Chlorophyta) – 1. Extaction and chemical composition. *Carbohydrate Research, 274*, 251–261.

Renn, D. W. (1992). In D. H. Attaway & O. R. Zaborosky (Eds.). *Marine biotechnology: Pharmaceutical and bioactive natural products* (Vol. 1, pp. 181–196). Plenum Press.

Skjak-Braek, G., & Martinsen, A. (1991). Applications of some algal polysaccharides in biotechnology. In M. D. Guiry & G. Blunden (Eds.), *Seaweed resources in Europe: Uses and potential* (pp. 219–256). John Wiley & Sons.

Stephen, A. M. (1995). *Food polysaccharides and their applications*. New York: Marcel Dekker.

York, W. S., Darvill, A., O'Neill, M., Stevenson, T., & Albersheim, P. (1985). Isolation and characterisation of plant cell walls and cell wall components. *Methods in Enzymology, 118*, 3–40.