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Polysaccharides from *Enteromorpha compressa*: Isolation, purification and structural features

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Abstract

Polysaccharides containing fractions were obtained from the seaweed *Enteromorpha compressa* by sequential extractions with various solvents. Anion exchange chromatography (AEC) of the oxalate extracted fraction (OSP) yielded rhamnoxylogalactoglucuronan, xyloglucan and glucuronan rich sub-fractions. The heteroglycuronan, which is sulfated, has an apparent molecular mass of 50 ± 20 kDa and is soluble in water. Methylation analysis of the native, desulfated and desulfated reduced heteroglycuronan sulfate suggested that this polymer is branched and contains, *inter alia*, (1 → 4)- and (1 → 2,4)-linked-rhamnopyranosyl, (1 → 4)-linked xylopyranosyl, and (1 → 4)- and terminally linked glucuronosyl residues. Sulfate groups, when present, are located at C-3 of (1 → 4)-linked rhamnose units and C-2 of (1 → 4)-linked xylose units. Degradation of the xyloglucan rich extract (Ec1OH) by *endo*-(1 → 4)-β-D-glucanase and analysis of the resulting fragments by matrix-assisted laser desorption ionization-time of flight-mass spectrometry (MALDI-TOF-MS) showed that the *E. compressa* xyloglucan contains Glc_3Xyl_1, Glc_4, $Glc_3Xyl_2(SO_3Na)_2$, $Glc_4Xyl_2(SO_3Na)_2$ and $Glc_5Xyl_2(SO_3Na)_2$ as major oligomeric building sub-units.

Keywords: *Enteromorpha compressa*; Sulfated heteroglycuronan; Xyloglucan sulfate; Glucuronan; Molecular mass; Linkage analysis; Enzyme hydrolysis; Oligosaccharides; MALDI-TOF-MS

1. Introduction

Seaweeds have been used widely for centuries traditionally only in Asia and marginally in the rest of the world mostly as food (Darcy-Vrillon, 1993; Indergaard & Minsaas, 1991). But in western countries, these are essentially used for the production of valuable chemicals and polysaccharides are the major chemical compound. The uses of these carbohydrate polymers span from food, cosmetic and pharmaceutical industries to microbiology and biotechnology (Franz, Pauper, & Alban, 2000; Gunay & Linhardt, 1999; Lewis, Stanley, & Guist, 1988; Skjak-Braek & Martinsen, 1991; Witvrouw & De Clercq, 1997).

Marine green algae belonging to *Ulvale* are frequently involved in algal proliferation in eutrophicated coastal and lagoon waters (Fletcher, 1996). One particularly interesting feature of Ulvale is their richness in polysaccharides. These macromolecules retain heavy metals and several *Ulva* and *Enteromorpha* species are used as bio indicators of pollution (Favero, Cattalini, Bertaggia, & Albergoni, 1996; Haritonidis & Malea, 1995). Ways to use these biomass, besides compost (Cuomo, Perretti, Palomba, Verde, & Cuomo, 1995; Maze, Morand, & Potoky, 1993), methane production (Brian & Morand, 1997) or paper making (Nicolucci & Monegato, 1993) could be based on specific properties of their polysaccharides. In edible Ulvales, polysaccharides play a nutritional role as dietary fibre (Andrieux et al., 1998; Bobin-Dubigeon, Lahaye, & Bary, 1997; Lahaye et al., 1998) and from different genera they demonstrate biological activities (Ivanova et al., 1994; Lee, Hayashi, Hayashi, Sankawa, & Maeda, 1999; Maeda, Uehara, Harada, Sekiguchi, & Hiraoka, 1991; Noda, Amano, Arashime, & Nisizwa, 1990) or gelling abilities

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(Haug, 1976; Lahaye, Ray, Baumberger, Quemener, & Axelos, 1996). Many papers have been published on the chemical characterization of polysaccharides present in Ulva spp. For example, it is known that the cell wall of the marine green algae *Ulva "rigida"* is composed of a water-soluble sulfated heteroglycan (named "ulvan"), linear β-(1 → 4)-xyloglucan, glucuronan and cellulose (Lahaye & Ray, 1996; Percival & McDowell, 1967; Ray & Lahaye, 1995a; Ray & Lahaye, 1995b). Preliminary studies on *Enteromorpha* spp. cell-wall polysaccharides have been carried out during sixties and seventies (Dodson & Aroson, 1978; Cronshaw, Myers, & Preston, 1958; McKinnel & Percival, 1962; Medcalf et al., 1972; Percival & McDowell, 1967; Yamamoto, 1980). But the structures of polysaccharides present in *Enteromorpha compressa*, another important member of Ulvale, have not yet been fully characterized. Therefore, the purpose of this work was to isolate, purify and characterize the polysaccharides present in this marine green alga.

2. Experimental

2.1. Plant material

Enteromorpha compressa (Linnaeus) Greville was kindly provided by (Dr. Marc Lahaye, INRA, France). The seaweed was heated in a current of hot air and ground to flour in a Waring Blender.

2.2. Extraction of pigments

The flour was depigmented by sequential extraction with organic solvents in a Soxhlet apparatus as described (Ghosh, Adhikari, Ghosal, Damonte, & Ray, 2004) except that acetone (48 h) and ethanol (48 h) were used instead of benzene (20 h), acetone (20 h) and methanol (20 h) used in earlier study. The resulting depigmented algal powder has been designated as DAP.

2.3. Sequential extraction with various inorganic solvents

The depigmented alga (DAP) was extracted sequentially with oxalate, and 1 and 4 M KOH solutions as described below.

2.3.1. Oxalate extraction

Extractions, at extractant (100 mM ammonium oxalate in 50 mM NaOAc buffer, pH 5) to algal flour (3 g) ratio of 150:1, were conducted in 1 L glass beakers stirred by a small propeller stirrer (1 h at 80 °C). Separation of the unextracted residue from the liquid was performed using a centrifuge followed by filtration of the centrifugate through fritted glass filter (G-3). The residue was briefly washed with additional extractant and the wash liquid was collected as well to maximize polysaccharide recovery. Here, washing was especially important, as the seaweed retain a large amount of residual solution. Next, the unextracted residue was extracted once more using similar condition. The combined extracts and washings were concentrated, dialyzed, and the concentrated retentate was diluted with 3-vol ethanol. The precipitate recovered by centrifugation as washed thoroughly with aqueous 80% and dry ethanol, acetone, and finally dried over P_2O_5 in vacuum to yield the oxalate soluble polymer (OSP; yield 780 mg).

2.3.2. Alkali extraction

The oxalate unextractable residue (OUR) was sequentially extracted with 1 and 4 M KOH following the procedure of Carpita (1984) as described (Mazumder, Lerouge, Loutelier-Bourhis, Driouich, & Ray, 2005). Briefly, the oxalate unextractable residue was suspended in 300 mL of 1 M KOH solution containing 300 mg $NaBH_4$ and the mixture was stirred continuously for 16 h at 4–8 °C. The suspension was filtered and the residual solid extracted once more with 300 mL of fresh extractant for 6 h at 30–35 °C. The combined viscous supernatant was brought to pH 5 by drop wise addition of acetic acid in an ice bath. The concentrated supernatant solution was dialyzed, concentrated and freeze dried (Ec1OH).

The residual weed was then extracted similarly with 4 M KOH to yield Ec4OH fraction. The resulting insoluble residue was washed thoroughly with deionised water, ethanol, and acetone and then dried over P_2O_5 in vacuum (EcINS).

2.4. Sugar analysis

Total sugars were determined by the phenol–sulfuric acid analysis using rhamnose as standard (Dubious, Gilles, Hamilton, Rebers, & Smith, 1956). Uronic acids were analyzed colorimetrically by the *m*-hydroxy diphenyl analysis according to procedure outlined by Ahmed and Labavitch (1977) using glucuronic acid as standard. Polysaccharide fraction were hydrolyzed either with 1 M sulfuric acid (3 h at 100 °C) for soluble substances or by treatment with 72% (w/w) H_2SO_4 for 1 h at room temperature and then with 1 M sulfuric acid for 3 h at 100 °C for water insoluble residues. *Myo*-inositol was used as internal standard. The released monosaccharide residues were converted into their alditol acetate (Blakeney, Harris, Henry, & Bruce, 1983) and analyzed by GLC and GLC/MS (Shimadzu QP 5050A) using DB-225 (JW). Uronic acid in the sulfuric acid hydrolysate was identified by TLC as previously described (Ghosh et al., 2005). Alternatively, the samples were hydrolyzed using trifluoroacetic acid (2 M, 2 h at 110 °C), followed by an 18 h methanolysis at 80 °C with dry 2 M methanolic–HCl. The generated methyl glycosides were converted into their TMS-derivatives and separated by gas chromatography (York, Darvill, O'Neill, Stevenson, & Albersheim, 1985). The gas chromatograph was equipped with a flame ionization detector, a WCOT fused silica capillary column (length 25 m, i.d. 0.25 mm) with CP-Sil 5 CP as stationary phase and helium as gas vector. The oven temperature program was: 2 min at 120 °C, 10 °C/min to 160 °C, and 1.5 °C/min to 220 °C, and then 20 °C/min to 280 °C.

2.5. Protein and Amino acid analysis

Proteins were estimated by colorimetry (Lowry, Rosebrough, Lewsfarr, & Randall, 1951). Amino acids were released by hydrolysis with 6 M HCl at 110 °C for 22 h in a sealed tube and were analyzed as described (Mazumder, Morvan, Thakur, & Ray, 2004).

2.6. Infra red spectroscopy

Infrared spectra of polysaccharides were recorded on a JASCO-FTIR 420 infrared spectrophotometer. Samples were dried at 35–44 °C in vacuum over P_2O_5 for 48 h prior to making pellet.

2.7. Anion-exchange chromatographic method

The oxalate soluble fraction (OSP) was purified by anion-exchange chromatography as described (Mazumder et al., 2004; Ray & Lahaye, 1995b). The crude polysaccharides were applied to a DEAE–Sepharose FF (acetate form) column (21 × 4 cm) equilibrated previously with 0.05 M sodium acetate buffer, pH 5.5. The column was washed with the same buffer (400 ml: fraction OF1) and then eluted successively with 400 ml of 0.2 M (fraction OF2), 0.7 M (fraction OF3) and 1.5 M NaOAc buffer pH 5.5 (fraction OF4) in a stepwise manner. Fractions (40 ml) were collected and analyzed for their total sugar and uronic acid contents. Appropriate fractions were concentrated, dialyzed against water, and finally lyophilized.

2.8. Gel-filtration chromatography

Size exclusion chromatography of the heteroglycan sulfate (OF4) on Sephadex G-200 column (90 × 2.6 cm) using 500 mM sodium acetate buffer (pH 4.0) as eluant was done as described (Mazumder et al., 2005). The column was calibrated with standard dextran sulfates (50 and 8 kDa; Sigma Chemical Company) and dextrans (10–500 kDa; a gift from Dr. Tapani Vuorinen).

2.9. Desulfation

Sulfate content was determined as described previously (Ghosh et al., 2004). Polysaccharides (OSP and OF4; 150 mg) were desulfated according to Nagasawa, Inoue, and Kamara (1977) and the desulfated polysaccharides (OSP-D and OF4-D, respectively) were isolated by freeze-drying (62 and 71 mg).

2.10. Reduction of uronic acid

Uronic acid in the desulfated polysaccharides (OF4-D) dissolved in 8 M urea (10 mg mL^{-1}, 10 mL) was reduced as described (York et al., 1985).

2.11. Methylation of the polysaccharides

Methylation analysis of polysaccharides (OF4, OF4-D and OF4-DR) by the modified Hakomori method (Blakeney & Stone, 1985) was carried out as previously described (Ray & Lahaye, 1995b).

2.12. Preparation of xyloglucan derived oligosaccharides

Sample (Ec1OH, 2 mg) was dissolved in 2 ml of 100 mM NaOAc (pH 5.0) and incubated with 40 U of *endo*-(1 → 4)-β-D-glucanase (Megazyme International, Ireland) for 24 h at 37 °C with constant shaking. Forty units of additional enzyme were added to the digest and incubation continued for a further 24 h. The digest was diluted with 4 vol of cold 95% ethanol, centrifuged and the resulting supernatant was concentrated to a small volume under nitrogen at 40 °C, and finally lyophilised. In this manner, xyloglucan oligosaccharides (1XGose) were generated from Ec1OH. Glucanase-resistant fraction, designated as 1GRF was obtained by lyophilisation of the ethanol insoluble residue.

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

Sample (2 μl) was mixed with 2 μl of the matrix solution (2,5-dihydroxybenzoic acid 10 mg/ml in TFA: CH_3CN: 1.75: 0.75; v/v) and a total of 2 μl of this solution was applied to a stainless steel sample slide and dried under vacuum. MALDI-TOF mass spectra of the generated oligomers were recorded on a Micromass (Manchester, UK) TOF spec E MALDI-TOF mass spectrometer. Spectra were acquired in the reflectron mode.

3. Results and discussion

3.1. Composition of depigmented algal powder and its polysaccharides after sequential extraction

The procedures used for the isolation and purification of polysaccharides from the marine green alga *E. compressa* are shown in Fig. 1, while yields and chemical composition are shown in Table 1. The depigmented algal powder (DAP) amounted to 80% of the starting algal dry weight and contained rhamnose, glucose, xylose and uronic acid residues (Table 1). But, after Saeman's hydrolysis, glucose was the main sugar released from DAP and, therefore, a part of the glucose was of cellulosic origin.

About 23% of DAP and 52% of its uronic acids were extracted by ammonium oxalate (OSP). These extracted polysaccharides were held in the walls by ionic cross-links, as chelating agents were able to extract calcium ions (Mathew, Howson, Keenan, & Belton, 1990). The oxalate-soluble fraction (OSP) is soluble in water and contained 53% carbohydrate on the basis of fraction dry weight. This fraction contained 8% of sulfate and residues of glucuronic acid, rhamnose, glucose, xylose, galactose

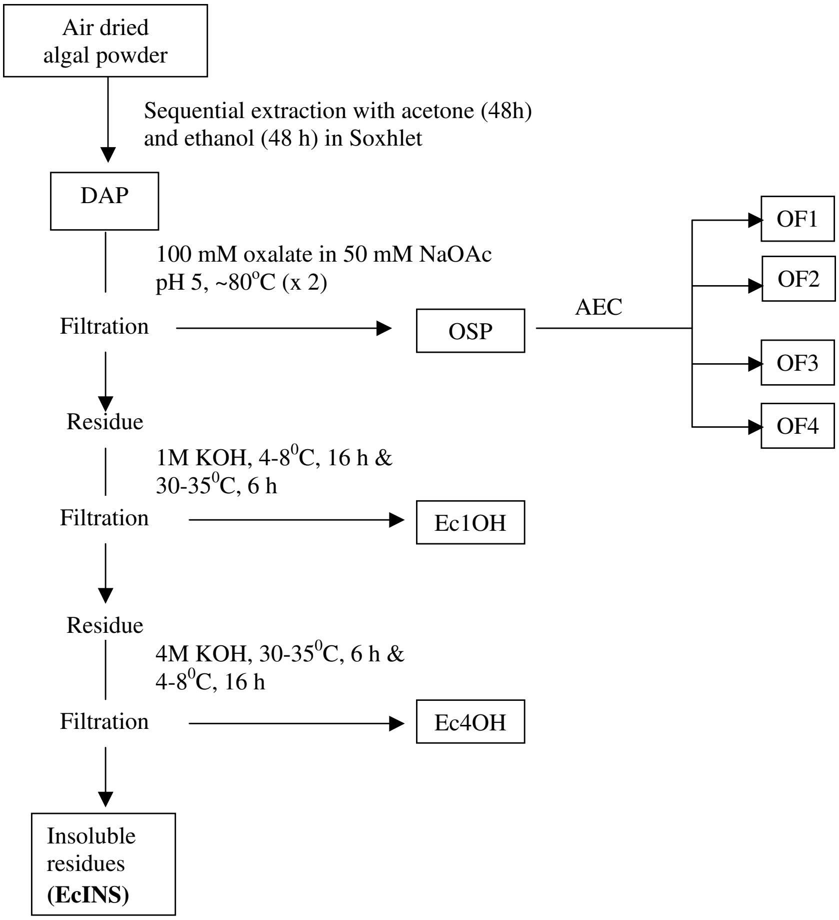

Fig. 1. Scheme for the isolation and purification of polysaccharides from *Enteromorpha compressa*.

Table 1
Yields and chemical composition of fractions obtained by sequential extraction of depigmented algal powder (DAP) of *Enteromorpha compressa* with various inorganic solvents (see text for the identification of fractions)

	DAP[d]	OSP	OSP-D	OUR	Ec1OH	Ec4OH	EcINS[d]
Yield[a]	100	23		56	8	9	28
TS[b]	45	53	89	nd	54	42	58
UA[b]	8	18	33	nd	11	9	6
Sulfate[b]	8	13	2	nd	6	4	nd
Protein[b]	nd	12	nd	nd	22	12	nd
Rha[c]	37 (2)	62	60	30	13	5	22 (1)
Ara[c]	tr (–)	–	2	–	–	–	1
Xyl[c]	16 (2)	15	13	20	17	25	11 (1)
Man[c]	4 (4)	tr	–	1	tr	tr	1 (4)
Me-Hex[c]	3 (–)	4	2	tr	8	–	– (–)
Gal[c]	7 (–)	4	7	1	–	–	1 (1)
Glc[c]	33 (92)	15	16	48	62	70	64 (93)

nd, not determined; –, not detected; tr, trace; TS, total sugar and UA, Uronic acid.

[a] Percent weights of depigmented alga dry weight.

[b] Percent weight of the fraction.

[c] Mole % of anhydro sugar.

[d] Values in the parentheses were obtained after Saeman's hydrolysis.

and methyl hexose (Table 1). Both, TLC analysis of the sugar released during hydrolysis and GLC analysis of the TMS-derivatives of the generated methyl glycosides, confirmed the presence of glucuronic acid. The sugar composition of this fraction (OSP) is similar to ulvan, the sulfated heteroglycan of *Ulva rigida* (Ray & Lahaye, 1995a), except that the former contains significantly higher amount of glucose and galactose residues. The protein content of OSP fraction was 12% (w/w) and the abundant amino acids were glutamic acid/glutamine and aspartic acid/asparagine (Table 2). Other prominent amino acids were glycine and alanine.

The hemicellulosic polysaccharides were isolated from the oxalate unextractable residue (OUR) using 1 and 4 M KOH as extractant. The yield of polymer extracted by the 1 M KOH was 8% of DAP. This fraction, Ec1OH, contained substantial amounts of glucose, xylose, rhamnose and methyl-hexose residues (Table 1). Uronic acid residues accounted for 11% (w/w) of Ec1OH and 11% of the uronic acids of DAP.

Other hemicellulosic polysaccharides, probably cross-linked within the wall matrix (Thompson & Fry, 2000), were extracted with 4 M KOH. The polysaccharide content of this alkali-extracted material (Ec4OH) was 42%, of which the major sugar residue was glucose (Table 1). Xylose, rhamnose and arabinose were the other neutral sugars present in this fraction. Previous studies (Lahaye et al., 1996; Ray & Lahaye, 1995a) on the hemicellulosic

Table 2
Amino acid composition of oxalate soluble fraction (OSP) obtained from the green alga *Enteromorpha compress*

Amino acid	Mole (%)
Aspartic acid/asparagines	16
Threonine	5
Serine	4
Glutamic acid/glutamine	20
Proline	3
Glycine	12
Alanine	10
Valine	6
Isoleucine	2
Leucine	5
Tyrosine	2
Phenylalanine	3
Histidine	1
Lysine	3
Arginine	8

polysaccharides of *U. rigida*, another *Ulvale*, showed the presence of xyloglucan. The fact that glucose and xylose together accounted for >68% of the neutral sugars of the alkali-extracted fractions (Ec1OH and Ec4OH) also suggest the presence of xyloglucan type polysaccharides in *E. compressa*. But, the glucose content of these alkali extracted fractions is higher than that of the hemicellulosic polymers of *U. rigida*.

The final residue (EcINS) amounted to 28% of DAP and contained 64 mol % glucose. But after Saeman's hydrolysis the amount of released glucose was increased. Therefore, the alkali insoluble residues from *E. compressa* contained cellulose. In total, ∼40% of the DAP could be extracted using sequential extraction with inorganic solvents.

The yields and sugar compositions of fractions obtained from *Ulvales* of different places are quite different (Dodson & Aroson, 1978; Cronshaw et al., 1958; McKinnel & Percival, 1962; Percival & McDowell, 1967; Medcalf et al., 1972; Ray & Lahaye, 1995a). But, it is well known that seasonal variations and the changes in pH, temperature etc. of places 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.

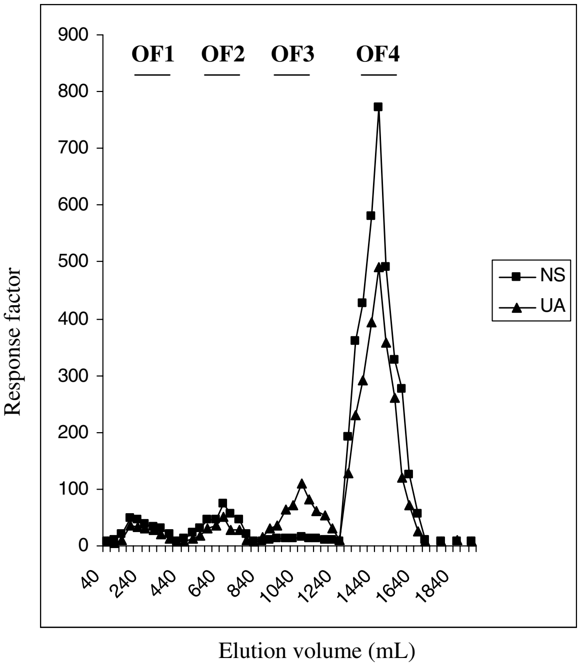

Fig. 2. Purification of the oxalate extracted fraction (OSP) from *Enteromorpha compressa* by anion exchange chromatography on DEAE–Sepharose column. The sub-fractions indicated by horizontal bars were pooled, dialysed, and lyophilised. NS, neutral sugar. UA, uronic acid.

Table 3
Sugar composition of sub-fractions recovered from anion exchange chromatography of the oxalate soluble fraction (OSP) and their derivatives (see text for identification of fractions)

	OF1	OF2	OF3	OF4	OF4-D	OF4-DR
NS[a]	6	9	3	82		
Glc-A[b]	6	7	18	69		
NS[c]	46	38	7	35	56	74
Glc-A[c]	1	2	18	16	26	2
Sulfate[c]	nd	nd	nd	17	2	nd
Rha[d]	tr	tr	13	67	68	48
Ara[d]	1	5	6	tr	1	–
Xyl[d]	12	27	36	28	26	16
Me-Hex[d]	–	–	–	1	2	tr
Man[d]	–	19	28	–	–	1
Gal[d]	–	7	6	1	1	tr
Glc[d]	87	42	11	3	2	35

nd, not determined; –, not detected and tr, trace.

[a] Weight percentage of total neutral sugar (NS) recovered.

[b] Weight percentage of total glucuronic acid (UA) recovered.

[c] Weight percent of fraction dry weight.

[d] Percentage mol.

3.2. Anion-exchange chromatography

The oxalate extracted fraction (OSP) was purified by anion exchange chromatography on DEAE–Sepharose (Fig. 2). The amount of polymers isolated from the column and the sugar composition of each fraction are given in Table 3. Pool 1 (OF1) consisted of unbound polysaccharides, and, as expected, had low amount of glucuronic acids (1 mol %). The amount of glucuronic acid increased with the ionic strength of the eluent. The fraction OF2, which was eluted at the beginning of the ionic gradient, was obtained in low amount. This pool OF2 consisted mostly of xylose and glucose residues indicating the presence of xyloglucan. But, the xylose to glucose ratio of this polysaccharide is very different from the xyloglucan present in higher plants (Carpita & Gibeaut, 1993; McNeil, Darvill, Fry, & Albersheim, 1984). In addition, this fraction also contained residues of mannose, rhamnose, galactose, arabinose and glucuronic acid and 21% protein (w/w). In the major fraction OF4, rhamnose alone accounted for 67% of the total neutral sugars. It also contained glucuronic acid (16% w/w) and sulfate (17% w/w). The chemical composition of this fraction is similar to rhamnoxylogalactoglucuronan obtained from *U. rigida* (Ray & Lahaye, 1995a). Apart from rhamnoxylogalactoglucuronan and xyloglucan, the presence of an acidic homoglucuronan, able to bind to the anion exchanger, was detected in OF3 fraction, where glucuronic acid was the most abundant sugars.

3.3. Size exclusion chromatography

Size exclusion chromatography of the heteroglycuronan sulfate on Sephadex G-200 suggests that the polymer is homogeneous. Based on calibration with standard dextrans, the apparent molecular weight of the polysaccharide present in OF4 would be 50 ± 20 kDa. It should, however, be noted that polysaccharides containing sulfate groups, due to intramolecular electrostatic repulsions by charge effects, may have a different hydrodynamic volume than dextrans and, therefore, elute at a different rate than expected on the basis of their molecular weight.

3.4. Desulfation and reduction

The oxalate soluble polysaccharide (OSP) and the purified sulfated heteroglycuronan (OF4) upon solvolytic desulfation yield desulfated polysaccharides (OSP-D and OF4-D, respectively) in 41–47% yield. OF4-D composed mainly of rhamnose, xylose, glucose together with smaller quantities of galactose and arabinose, and glucuronic acid (Table 3). The desulfated and then reduced polymer (OF4-DR) showed a marked increase in glucose content, thus glucuronic acid is the uronic acid of this heteroglycuronan. The very similar sugar composition of the native (OF4) and the desulfated polysaccharide (OF4-D) indicates that the backbone of former was unaffected by desulfation. Interestingly, the sugar composition of the desulfated (OF4-D) and desulfated-reduced (OF4-DR) fractions is similar to the desulfated, and then reduced polymer derived from *U. rigida* (Ray & Lahaye, 1995a).

3.5. Linkage analysis

Methylation of the sulfated heteroglycan containing fraction (OF4) of *E. compressa* yielded a variety of methylated derivatives Table 4. Despite the observation that the proportions of methylated derivatives remain unchanged after an additional methylation cycle (data not shown), it is probable that there was incomplete methylation. In fact, methylation of sulfated 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 sulfated heteroglycan (OF4) and its derivatives (OF4-D and OF4-DR) suggest that the rhamnoses are terminal, (1 → 4)- and (1 → 2,4)-linked. Xyloses are in pyranose form and are (1 → 4)-linked. *O*-2 of xylose and *O*-3 of rhamnose was the site of ester sulfate. Glucuronic acids are (1 → 4)- and terminally linked. The presence of (1 → 3)- and (1 → 6)-linked galactosyl, and (1 → 4)- and terminal glucosyl residues are also indicated. Thus, this acidic heteroglycan is branched and the linkage pattern of this sulfated polymer is similar to that of ulvan i.e., the sulfated heteroglycan from *U. rigida* (Ray & Lahaye, 1995a).

Table 4
Linkage analysis of the constituent sugars of the native (OF4), desulfated (OF4-D) and desulfated and then reduced (OF4-DR) heteroglycuronan of the marine green alga *Enteromorpha compressa*

Partially methylated alditol acetates[a]	Deduced units and substitution pattern	OF4	OF4-D	OF4-DR
2,3,4-Me$_3$-Rha	T[b]	1	1	1
3,4-Me$_2$-Rha	→2)-Rha	tr	1	tr
2,3-Me$_2$-Rha	→4)-Rha	2	50	39
2,4-Me$_2$-Rha	→3)-Rha	2	–	tr
2-Me-Rha	→4)-Rha 3 S[c]	48	1	tr
3-Me-Rha	→2,4)-Rha	tr	10	12
Rha	→2)-Rha	12	2	1
2,4,6-Me$_3$-Gal	→3)-Gal	1	tr	2
2,3,4-Me$_3$-Gal	→6)-Gal	3	1	tr
2,3,4,6-Me$_4$-Glc	T	2	1	5
2,3,6-Me$_3$-Glc	→4)-Glc	11	12	21
2,3-Me$_2$-Xyl	→4)-Xyl	11	18	16
3-Me-Xyl	→4)-Xyl 2 S[c]	5	1	1
Xyl		2	tr	tr

tr, trace; –, not detected.

[a] 2,3,4-Me-Rha, 2,3,4-tri-*O*-methyl-1,5-di-*O*-acetyl rhamnitol etc.

[b] T, terminal.

[c] S, SO$_3^-$.

3.6. FT-IR

The FT-IR spectra of OSP, OSP-D, OF4, OF4-D, Ec1OH and Ec4OH fractions showed bands characteristics of polysaccharides (Kacurakova, Capek, Sasinkova, Wellner, & Ebringerova, 2000; Monsoor, Kalapathy, & Proctor, 2001). In addition, OSP, OF4, Ec1OH and Ec4OH fractions showed a band in the region 1240–1260 cm^{-1} related to >S=O stretching vibration of the sulfate group (Percival & Wold, 1963). Two other bands at 790 and 850 cm^{-1} arising from the sulfate group of polysaccharides were also observed. IR spectra of the desulfated polymers (OSP-D and OF4-D), on the other hand, showed reduction of these bands.

3.7. Hydrolysis with endoglycosidase

Sugar composition of Ec1OH indicates that this fraction contain xyloglucan type polysaccharides. But composition analysis by simple acid hydrolysis may yield ambiguous information (Fry, 1989). Therefore, further information on the structure of polymers present in xyloglucan-rich fraction (Ec1OH) was obtained by enzymatic degradation of this fraction and structural analysis of the resulting fragments. Ec1OH was submitted to a depolymerization with an *endo*-(1 → 4)-β-D-glucanase, an enzyme known to cleave β-(1 → 4)-glucosidic linkages of xyloglucan backbone next to an unbranched glucose residue (Fry, 1989). The endoglucanase-resistant fraction (named 1GRF) was removed from the digest by precipitation with 80% (v/v) ethanol. Endoglucanase-generated xyloglucan oligosaccharides (1XGose) were recovered from the supernatant of the digest. Sugar compositional analysis of 1XGose revealed that xylose (26 mol %) and glucose (74 mol %) were the major monosaccharides. Generation of oligosaccharides by *endo*-(1 → 4)-β-D-glucanase indicates the presence β-(1 → 4)-linked glucose residues in the polymer. Moreover, as both glucose and xylose residues are present in the enzyme generated oligosaccharides they are integral part of a single polysaccharide. Only glucose containing oligosaccharides would have been expected from linear glucan chain which would have also been insoluble in water.

MALDI-TOF mass spectrum (Fig. 3) of 1XGose reveals the presence of five oligosaccharides. On the basis of their molecular weight, pseudo molecular ions at *m/z* 659, 689, 995, 1157 and 1319, correspond to Hex$_3$Pent$_1$, Hex$_4$, Hex$_3$Pent$_2$(SO$_3$Na)$_2$, Hex$_4$Pent$_2$(SO$_3$Na)$_2$ and Hex$_5$Pent$_2$(SO$_3$Na)$_2$, respectively. Although mass spectroscopy cannot distinguish stereo isomers, but sugar compositional analysis indicates the presence of xylose and glucose residues in the enzyme derived oligosaccharides. Therefore, Hex$_3$Pent$_1$, Hex$_4$, Hex$_3$Pent$_2$(SO$_3$Na)$_2$, Hex$_4$Pent$_2$(SO$_3$Na)$_2$ and Hex$_5$Pent$_2$(SO$_3$Na)$_2$ could be assigned to Glc$_3$Xyl$_1$,Glc$_4$, Glc$_3$Xyl$_2$(SO$_3$Na)$_2$, Glc$_4$Xyl$_2$(SO$_3$Na)$_2$ and Glc$_5$Xyl$_2$(SO$_3$Na)$_2$, respectively. Appearance of pseudo molecular ion at *m/z* 689 corresponding to Glc$_4$ indicates the presence of a block containing four nonsulfated glucose residues.

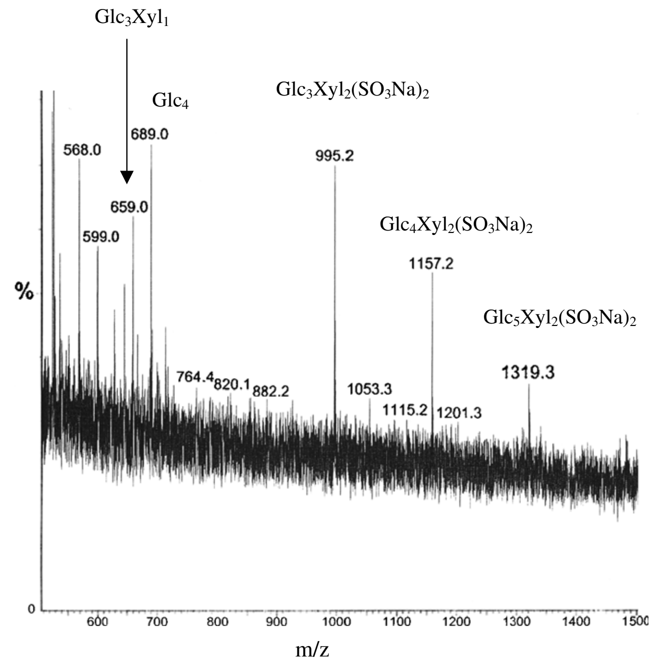

Fig. 3. MALDI-TOF-mass spectrum of 1XGose fraction generated by endo-glucanase treatment of the xyloglucan rich extract (Ec1OH) isolated from *Enteromorpha compressa.*

4. Conclusions

In conclusion, the marine green alga *E. ompressa* contains at least four different types of polysaccharides. The oxalate extracted heteroglycuronan is branched, sulfated and have linkage pattern similar to that of the sulfated heteroglycan of *U. rigida*. The presence of xyloglucan sulfate, glucuronan and cellulose were also indicated. The complete structure of the sulfated xyloglucan isolated by extraction with alkali was not obtained, but some important structural features were established. It was shown, for the first time that the xyloglucan of *E. compressa* could be degraded with *endo*-β-(1 → 4)-D-glucanase and, therefore, the backbone of this polymer contains β-(1 → 4)-linked glucopyranosyl residues. Besides, this polymer is sulfated, and posses a block containing at least four glucose residues. Moreover, as both glucose and xylose residues are present in some enzyme generated oligosaccharides they are the integral part of a single polysaccharide. Finally, this xyloglucan contains Glc$_3$Xyl$_1$, Glc$_4$, Glc$_3$Xyl$_2$(SO$_3$Na)$_2$, Glc$_4$Xyl$_2$(SO$_3$Na)$_2$ and Glc$_5$Xyl$_2$(SO$_3$Na)$_2$ as major oligomeric building sub-units. But, the distribution of sulfate groups along the polymer chain and the sequence of sugar residues are not known. Further work in this regard is in progress.

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