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Cell-wall polysaccharides from the marine green alga *Ulva "rigida"* (Ulvales, Chlorophyta). Extraction and chemical composition ☆

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Abstract

Cell-wall polysaccharides from the green seaweed *Ulva "rigida"* were extracted sequentially with oxalate, 1 and 4 M KOH, sodium chlorite and 4 M KOH again. The chemical composition of the soluble and insoluble extracts and of the DEAE-Sepharose CL-6B fractions of the soluble polysaccharide were determined. Three main types of polysaccharide families were solubilized. The major family was composed of sulfated glucuronorhamnoxyloglycans (ulvan) and was essentially extracted with oxalate. Two hemicellulosic fractions were also isolated and consisted of glucuronans and glucoxylans. Minor fractions consisting of sulfated polysaccharides containing glucose, xylose, mannose and protein were also isolated.

Keywords: *Ulva*; Chlorophyceae; Algae; Cell wall; Ulvan; Xyloglucan; Glucuronan

1. Introduction

The marine green seaweed genus *Ulva* is poorly utilized and only a small part of its biomass is used as edible seaweeds [1]. Periodically, particular species overgrow as "green-tides" in eutrophicated areas (Brittany, Venice lagoon) and create ecological and economical problems [2]. One particularly interesting feature of *Ulva* is their richness in cell-wall polysaccharides, which can be used for their functional and/or biological

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Cell-wall polysaccharides from the marine green alga *Ulva "rigida"* (Ulvales, Chlorophyta).

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properties in the making of compost [3] or paper [4], for their gelling ability [5,6] or their anti-viral activities [7]. As in other "sea vegetables", these polysaccharides contribute to the nutritional benefits of these seaweeds as dietary fibre [8]. However, the full use of *Ulva* cell-wall polysaccharides requires an in-depth knowledge of the relationships between their structures and properties. To date, most studies concern extraction [9,10] composition and structure [11–18] and gellation properties [5,6] of the water-soluble polysaccharides. These were reported as ramified sulfated polysaccharides composed of xylose, glucuronic acid and rhamnose, occuring on the same chain as sulfated glucuronorhamnoxyloglycan [19] and for which the name ulvan has been proposed [6]. Reports on the water insoluble polysaccharides showed that the alkali-insoluble residues failed to give the cellulose-I type X-ray diffraction pattern [20–22] and contained linear β-1,4-linked xyloglucan [22]. Only after strong acid treatment of *Ulva* α-cellulose, was a cellulose-I X-ray diffraction pattern reported [23]. Sulfated glycoproteins, composed of glucuronic acid, glucose, rhamnose and xylose, were reported from 1 M NaOH extract [24] and xylose, glucose, and galactose composed the 4 M KOH extract of *Ulva lactuca* [20] but the exact nature of the hemicellulosic polysaccharides is unknown. This report is part of a larger study aimed at determining the different polysaccharide families in *Ulva* cell walls [25–27].

2. Experimental

Material.—Algae were collected at Piriac (Pointe du Castelli, Loire-Atlantique, France) in May 1993 and were tentatively identified as *Ulva "rigida"* from the morphology and collection site. The seaweeds were freed from attached impurities, washed thoroughly with tap water, dried in a current of air at 60°C and ground to a flour in a Waring Blender.

Extraction of polysaccharides.—The algal flour was extracted sequentially with hot sodium oxalate solution, hot water, 1 M and 4 M KOH solution at 20°C.

Sodium oxalate extraction.—The dried weed (372 g) was suspended in 3.8 L of 0.05 M sodium oxalate solution (pH 6) and stirred for 1.5 h in a boiling water bath. The suspension was diluted by the addition of 3 L of sodium oxalate solution prior to centrifugation (20 min, 14,740 *g*). The residue was re-extracted with 3.8 L of boiling sodium oxalate for a further 1 h and then with 5 L of boiling deionized water for 2 h. The last slurry was filtered through a fritted-glass G-4 filter (porosity 5–15 μm) and the residue washed thoroughly with deionized water. The combined extracts (sodium oxalate and water) and water washings were ultrafiltered (Mw cutoff 30 kDa, Amicon hollow fibre cartridges) and the retentate (3 L) was precipitated in 3 vol 95% ethanol. The precipitate was recovered by centrifugation (20 min, 14 740 *g*), washed thoroughly with 80% ethanol, 95% ethanol, and acetone, and finally dried over P_2O_5 in vacuo (A; yield 76.4 g).

The residue was extracted sequentially at room temperature with 3 L of 60%, 80%, 95% ethanol and acetone. The pale-yellow solid (B) was air-dried and then dried in vacuo over P_2O_5 (yield 142 g).

Alkaline extract.—Extraction with 1 M and 4 M KOH solution were performed

following the procedure of Carpita [28]. The residual weed (B, 114 g) was suspended in 2 L of 1 M KOH solution containing 0.3% $NaBH_4$ (w/v) and stirred for 1.5 h at 20°C under N_2. The suspension was centrifuged (15 min, 14740*g*) and the residual solid extracted once more in similar conditions with 1.5 L of fresh extractant. The combined viscous supernatants with 2 drops of octanol was brought to pH 4.5 by the slow addition of 6 M HCl in an ice-bath. After 3 h, the precipitate formed during acidification was removed by centrifugation (15 min, 14740*g*). The concentrated supernatant solution was then treated with α-amyloglucosidase from *Aspergillus niger* (300 mg, Merck) for 6 h at room temperature followed by 14 h at 4°C, and then dialysed, concentrated, and freeze-dried (S1; yield 3.9 g).

The residual weed (1 M KOH insoluble residue) was then extracted similarly with 4 M KOH to yield S4 (10.4 g). The resulting insoluble residue was washed thoroughly with deionized water, 95% ethanol and acetone, air-dried and then dried over P_2O_5 in vacuo (OHINS; yield 47.1 g).

Chlorite treatment.—OHINS was then treated with $NaClO_2$–AcOH as described [29], with a shorter treatment time. To OHINS (10 g) in deionized water (400 mL) at 70°C and under N_2 portions of acetic acid (4 × 1 mL) and sodium chlorite (4 × 2.5 g) were added at 15 min intervals. After 1 h of reaction the slurry was filtered through a fritted-glass G-3 filter (porosity 15–40 μm) and the residue was washed with 2 L of deionized water. The combined extract and washing was purged with N_2, adjusted to pH 6.8, concentrated, dialysed and freeze-dried (NC; yield 1.97 g). The $NaClO_2$-insoluble α-cellulose was then re-extracted with 4 M KOH containing 0.3% $NaBH_4$ (1 L) and the suspension filtered through a G-3 filter. The residue was washed first with acidified deionized water (500 mL) until the washings reached pH 6.5 and then sequentially with deionized water (1.5 L), 95% ethanol, and acetone. The final residue was dried in vacuo, over P_2O_5 (INS; yield 4.6 g). The alkaline extract was adjusted to pH 5.5 by the slow addition of glacial acetic acid, concentrated, dialysed and freeze-dried (S*4; yield: 0.95 g).

Anion exchange chromatography.—Fractions A, S1, S4, NC and S*4 (30–35 mg) dissolved in deionized water (20 mL) were applied to a column (1.6 × 23 cm) of DEAE-Sepharose CL-6B (Cl^- form, Pharmacia). Elution (0.5 mL min^{-1}) was with water (100 mL), then with a linear gradient of (0–> 1 M) NaCl (500 mL) followed by 150 mL of 1 M NaCl. Fractions (5 mL) were analysed for sugar and uronic acid contents. Appropriate fractions were pooled, concentrated, dialysed (if necessary), and freeze-dried.

Chemical analysis.—Ash was determined by incineration overnight at 550°C followed by 2 h at 900°C. Protein contents of the insoluble and soluble material were estimated by the micro Kjelhdahl digestion procedure ($N \times 6.25$) and the Lowry method [30], respectively. Sulfate contents were determined in 5–10 mg material after hydrolysis with 2 M trifluoroacetic acid for 3 h at 100°C and HPLC analysis of the hydrolysate on a Nucleosil Anion II column (Macherey-Nagel, Düren, Germany) as described [6]. Uronic acid contents were analysed colorimetrically by the automated *m*-phenyl phenol method using glucuronic acid standard [31]. Neutral sugars were determined after hydrolysis with 2 M H_2SO_4 for 2 h at 100°C, preceeded by a 30 min prehydrolysis at 20°C with aqueous 72% H_2SO_4 for insoluble residues [32]. Reduction and acetylation

were carried out as described [33] and the alditol-acetates were analysed by GLC using a DB 225 fused sillica capillary column (JW Scientific) operating isothermally at 210°C with H_2 as carrier gas.

GLC-mass spectrometry.—GLC-MS analysis of acetylated alditols was carried out using the same GLC chromatographic system as above coupled to a R10-10C Delsi-Nermag mass spectrometer operating in EI mode at 70 eV with a transfer chamber temperature of 250°C.

3. Results

The yield and chemical composition of *Ulva "rigida"* polysaccharides extracted sequentially with hot ammonium oxalate, 1 M and 4 M KOH, sodium chlorite and once more with 4 M KOH solutions are given in Table 1. The oxalate fraction (A) amounted to 20.0% of the starting algal dry weight and contained 51.2% polysaccharides on the basis of the fraction dry weight. It was mainly constituted of rhamnose, uronic acid, xylose and sulfate (Table 1) and was fractionated on DEAE-Sepharose CL-6B into one neutral (Aaq.) and four charged (AF1, AF2, AF3 and AF4) fractions (Fig. 1A). The recovery yield from the anion exchanger was 102.7% on the total sugar basis and the yields and chemical compositions of the fractions are given in Table 2. The major fraction, AF4, which accounted for 78.0% and 83.3% of the neutral sugars and uronic acids recovered from the anion exchanger, respectively, had a composition close to that

Table 1
Chemical composition of the cell-wall material extracted from *Ulva "rigida"* (see text for identification of fractions)

	A	B	S1	S4	OHINS	NC	S*4	INS
Yield [a]	20.0	38.5	1.3	3.5	15.8	3.1	1.5	7.2
Neutral sugar [b]	30.3	32.9	48.7	31.8	49.3	34.9	40.7	59.0
Uronic acid [b]	20.9	7.4	11.8	14.6	10.7	24.3	10.0	9.3
Protein [b]	13.9	12.4	25.9	13.2	5.9	5.8	14.3	2.8
Ash [b]	25.0	19.2	8.1	17.2	19.3	12.3	13.0	16.0
Sulfate [c]	35.9	20.4	17.8	27.3	18.0	26.5	21.2	14.4
Uronic acid [c]	24.9	12.9	15.2	19.9	13.1	27.2	13.7	10.3
Rhamnose [c]	23.3	10.8	17.6	19.5	10.7	20.6	12.4	11.3
3/4-O-Me hexose [c]	1.6	tr [d]	tr	0.6	tr	nd	nd	nd
Arabinose [c]	nd	tr	tr	0.9	0.5	1.1	1.1	0.7
Xylose [c]	10.9	14.5	33.3	21.2	11.0	9.1	15.6	17.9
Mannose [c]	tr	tr	1.7	0.9	0.7	nd	nd	nd
Galactose [c]	0.4	tr	tr	0.3	tr	tr	tr	nd
Glucose [c]	3.0	41.3	19.1	9.5	46.1	15.5	36.0	45.4

[a] Percentage weight of alga dry weight.
[b] Percentage weight of the fraction.
[c] Percentage mol.
[d] tr: trace.
[e] nd: not detected.

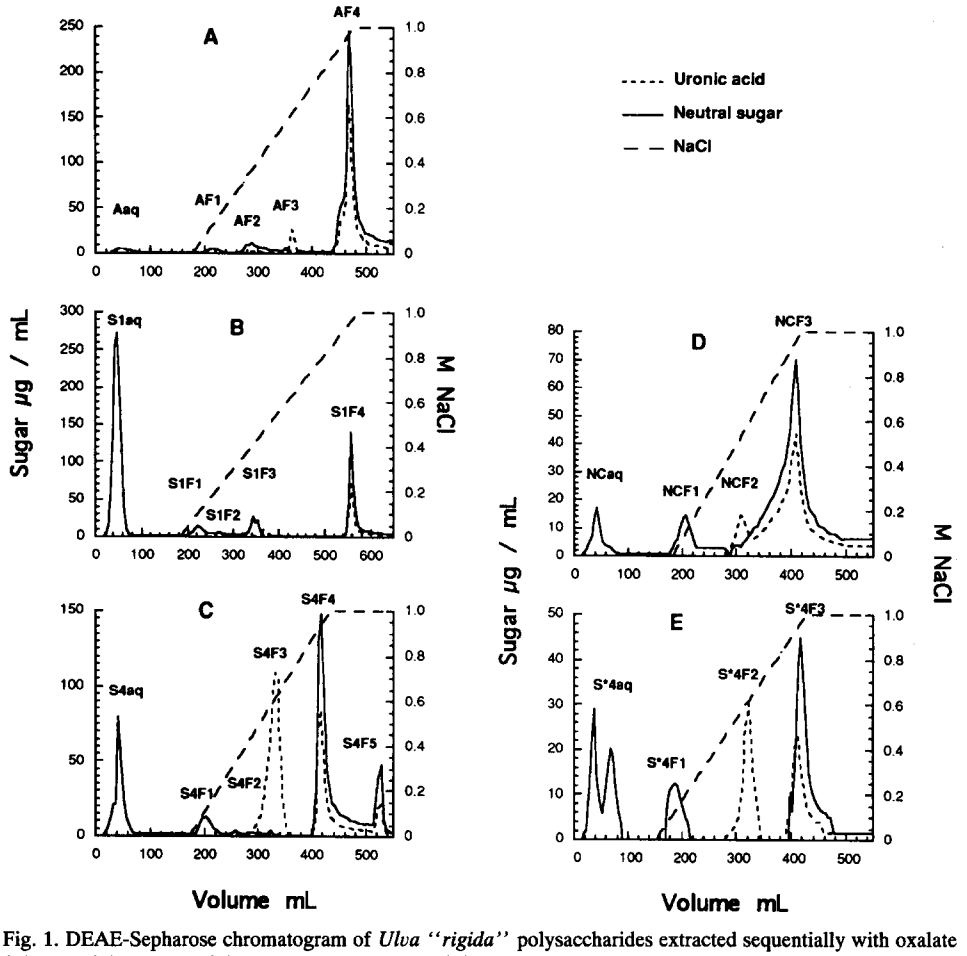

Fig. 1. DEAE-Sepharose chromatogram of *Ulva "rigida"* polysaccharides extracted sequentially with oxalate (A), 1 M (B) and 4 M (C) KOH, sodium chlorite (D) and a second time with 4 M KOH (E).

of the whole extract (A) with rhamnose, uronic acid, xylose and sulfate (Table 2). The oxalate insoluble residue (B) represented 38.5% of the dry seaweed and contained 40.3% polysaccharides composed mostly of glucose with smaller amounts of xylose, rhamnose, uronic acids and sulfate (Table 1). Sequential extraction of B with 1 M and 4 M KOH solubilized polymers that accounted for 2.1 and 6.4% of the dry seaweed weight, respectively. After acidification of the alkaline extracts, 1.3 and 3.5% of the dry seaweed weight was recovered in the soluble fractions S1 and S4, respectively (Table 1). S1 and S4 contained 60.5 and 46.4% of polysaccharides, respectively, composed of xylose, rhamnose, uronic acid, glucose and sulfate (Table 1). S1 was richer in xylose and glucose and poorer in rhamnose, uronic acid and sulfate than S4 and yielded one major neutral (S1aq.) and four acidic fractions (S1F1, S1F2, S1F3 and S1F4) on DEAE-Sepharose chromatography (Fig. 1B). The recovery yield from the anion ex-

Table 2
Yield and chemical composition of fractions recovered from DEAE Sepharose CL-6B chromatography of the oxalate soluble material (see text for identification of the fractions)

	Aaq.	AF1	AF2	AF3	AF4
Yield NS [a]	2.4	2.0	6.2	1.1	78.0
Yield UA [b]	0	0	3.7	6.8	84.3
Uronic acid [c]	tr [e]	1.3	4.6	39.8	19.1
Total sugars [c]	47.2	59.4	26.4	59.1	49.5
Sulfate [c]	nd [f]	nd	nd	nd	19.2
Protein [c]	nd	28.0	34.4	19.7	3.3
Sulfate [d]	nd	nd	nd	nd	38.4
Uronic acid [d]	tr	1.9	14.7	63.5	20.9
Rhamnose [d]	—	18.6	10.3	4.7	26.3
3/4-O-Me hexose [d]	—	—	tr	1.7	1.6
Arabinose [d]	—	—	17.1	2.4	tr
Xylose [d]	11.9	15.1	24.7	12.6	11.6
Mannose [d]	—	23.3	19.0	9.6	1.3
Galactose [d]	—	—	6.1	1.6	tr
Glucose [d]	88.1	42.0	8.9	3.9	tr

[a] Percentage weight of total neutral sugar recovered.
[b] Percentage weight of total uronic acid recovered.
[c] Percentage weight of fraction dry weight.
[d] Percentage mol.
[e] tr: trace.
[f] nd: not determined.
—: not detected.

Table 3
Yield and chemical composition of the fractions obtained from DEAE Sepharose CL-6B chromatography of the S1 fraction (see text for identification of fractions)

	S1aq.	S1F1	S1F2	S1F3	S1F4
Yield NS [a]	57.6	3.7	1.2	5.7	20.7
Yield UA [b]	0	0	0.5	11.5	54.8
Uronic acid [c]	0.4	0.4	0.9	6.8	15.4
Total sugars [c]	50.9	44.9	11.0	12.8	50.0
Sulfate [c]	tr [e]	nd [f]	nd	nd	14.7
Protein [c]	18.6	26.0	71.0	61.4	4.9
Sulfate [d]	tr	nd	nd	nd	39.7
Uronic acid [d]	tr	0.8	7.0	48.5	22.6
Rhamnose [d]	2.4	7.4	tr	—	23.8
3/4-O-Me hexose [d]	—	—	—	—	tr
Arabinose [d]	—	tr	tr	—	—
Xylose [d]	54.5	35.0	32.5	57.1	12.0
Mannose [d]	tr	24.3	39.1	22.1	2.0
Galactose [d]	—	3.0	—	—	—
Glucose [d]	42.4	25.6	21.4	tr	—

[a] Percentage weight of total neutral sugar recovered.
[b] Percentage weight of total uronic acid recovered.
[c] Percentage weight of fraction dry weight.
[d] Percentage mol.
[e] tr: trace.
[f] nd: not determined.
—: not detected.

Table 4
Yield and chemical composition of the fractions obtained from DEAE sepharose CL-6B chromatography of the S4 fraction (see text for identification of fractions)

	S4aq.	S4F1	S4F2	S4F3	S4F4	S4F5
Yield NS [a]	17.6	5.8	1.4	2.0	51.2	10.8
Yield UA [b]	0	0	0.1	53.4	33.3	6.3
Uronic acid [c]	0.3	3.5	1.7	48.6	19.4	16.4
Total sugars [c]	71.4	68.7	14.8	51.9	51.8	47.2
Sulfate [c]	tr [e]	2.0	nd [f]	1.5	20.6	26.3
Protein [c]	4.5	12.3	56.3	23.1	2.3	nd [f]
Sulfate [d]	tr	4.2	nd	5.0	38.9	47.1
Uronic acid [d]	tr	4.1	9.9	87.9	20.0	16.0
Rhamnose [d]	tr	4.7	24.7	4.3	24.8	20.7
3/4-O-Me hexose [d]	—	—	tr	tr	1.0	—
Arabinose [d]	—	0.6	6.4	—	0.9	0.8
Xylose [d]	52.4	48.9	18.9	1.5	12.2	10.9
Mannose [d]	1.1	4.1	15.1	0.5	1.3	3.1
Galactose [d]	—	1.3	tr	0.8	tr	—
Glucose [d]	46.0	32.3	25.0	tr	0.8	1.4

[a] Percentage weight of total neutral sugar recovered.
[b] Percentage weight of total uronic acid recovered.
[c] Percentage weight of fraction dry weight.
[d] Percentage mol.
[e] tr: trace.
[f] nd: not determined.
—: not detected.

changer was 98.6% on the total sugar basis and fractions yield and composition are given in Table 3. S1aq. accounted for 57.6% of the neutral sugars recovered from the anion exchanger and was mainly composed of xylose and glucose. The composition of the major acidic fraction, S1F4, was similar to that AF4 (Table 2). Chromatography of S4 through DEAE-Sepharose (Fig. 1C) yielded three major fractions: one neutral (S4aq.) and two acidic fractions (S4F3 and S4F4) and three minor charged fractions (S4F1, S4F2 and S4F5) yield and composition are given in Table 4. The recovery yield from the anion exchanger was 102.2% on the total sugar basis. S4aq. and S4F4 had a sugar composition close to that of S1aq. and S1F4 (Table 3), respectively, whereas S4F3 was composed almost solely of uronic acid. The sugar composition of the alkali-insoluble residues (OHINS) was similar to that of the oxalate-insoluble residues (B, Table 1) and the removal of the residual proteins and the non-cellulosic components was attempted by aqueous sodium chlorite extraction. This treatment solubilized 3.1% of the alga dry weight in a fraction (NC) containing 59.2% of polysaccharides mainly composed of uronic acid, sulfate, rhamnose, glucose and xylose (Table 1). Because little protein was extracted with chlorite (5.8% of NC; Table 1), the residue was extracted once more with 4 M KOH to yield an α-cellulose (INS) slightly enriched in xylose (Table 1). The neutralized soluble alkaline extract (S*4) accounted for 1.5% of the dry seaweed weight and contained 14.3% proteins with 50.7% polysaccharides composed essentially of glucose, xylose, uronic acid, rhamnose and sulfate (Table 1). The anion exchange

Table 5
Yield and chemical composition of the fractions obtained from DEAE Sepharose CL-6B chromatography of the $NaClO_2$ extract (NC) (see text for identification of fractions)

	NCaq.	NCF1	NCF2	NCF3
Yield NS [a]	5.9	6.2	7.1	71.1
Yield UA [b]	0	0	15.3	79.2
Uronic acid [c]	tr [e]	0.7	6.8	16.7
Total sugars [c]	43.6	56.0	45.4	47.6
Sulfate [c]	tr	4.5	nd [f]	20.0
Protein [c]	1.0	3.3	7.4	4.0
Sulfate [d]	tr	11.8	nd	40.2
Uronic acid [d]	0.9	0.9	73.4	18.4
Rhamnose [d]	tr	tr	tr	25.0
3/4-O-Me hexose [d]	—	—	—	tr
Arabinose [d]	1.8	tr	—	1.0
Xylose [d]	57.5	7.4	7.5	10.2
Mannose [d]	—	9.8	9.3	2.4
Galactose [d]	9.3	2.3	—	tr
Glucose [d]	30.8	67.7	37.0	2.8

[a] Percentage weight of total neutral sugar recovered.
[b] Percentage weight of total uronic acid recovered.
[c] Percentage weight of fraction dry weight.
[d] Percentage mol.
[e] tr: trace.
[f] nd: not determined.
—: not detected.

Table 6
Yield and chemical composition of the fractions obtained from DEAE Sepharose CL-6B chromatography of the S*4 fraction (see text for identification of fractions)

	S*4aq.	S*4F1	S*4F2	S4*F3
Yield NS [a]	25.1	12.1	5.1	49.0
Yield UA [b]	0	0	35.7	15.0
Uronic acid [c]	0.5	0.5	34.5	15.0
Total sugars [c]	63.8	46.9	44.0	42.1
Sulfate [c]	tr [e]	6.5	4.0	14.7
Protein [c]	14.2	33.5	33.3	4.3
Sulfate [d]	tr	17.7	13.8	41.9
Uronic acid [d]	tr	0.7	64.9	20.0
Rhamnose [d]	1.1	—	tr	25.8
Arabinose [d]	tr	—	3.5	tr
Xylose [d]	17.1	37.0	7.0	12.3
Mannose [d]	tr	6.3	7.4	tr
Galactose [d]	tr	tr	tr	—
Glucose [d]	80.4	38.3	3.5	tr

[a] Percentage weight of total neutral sugar recovered.
[b] Percentage weight of total uronic acid recovered.
[c] Percentage weight of fraction dry weight.
[d] Percentage mol.
[e] tr: trace.
—: not detected.

chromatography of NC (Fig. 1D) yielded one neutral (NCaq.) and three acidic (NCF1, NCF2 and NCF3) fractions. The chemical and sugar compositions of these are reported in Table 5. The recovery yield from the anion exchanger was 88.9% on the total sugar basis. Similarly, the anion exchange chromatography of S*4 (Fig. 1E) yielded one neutral, S4*aq., and three acidic fractions, S*4F1, S*4F2 and S*4F3. Their chemical compositions are given in Table 6. The recovery yield from the anion exchanger was 95.1% on the total sugar basis. Although poorer in glucose and containing galactose, NCaq. composition was similar to that of S4F1 (Table 4). NCF1 was composed of glucose and sulfate and NCF2 contained glucose and was rich in uronic acid. S*4aq. contained mainly glucose with some xylose, whereas S*4F1 was composed of almost equimolar amounts of xylose and glucose with sulfate and some mannose. The uronic acid-rich S*4F2 fraction had a composition close to that of S4F3 (Table 4) with the addition of sulfate. The highly charged fractions NCF3 and S4*F3 had close chemical compositions resembling those of AF4, S1F4 and S4F4 (Tables 2–4).

4. Discussion

Three main types of soluble polysaccharides were extracted from the cell wall of *Ulva "rigida"*. The major population consisted of acidic polysaccharides recovered with similar NaCl concentrations from the anion exchange chromatography of the oxalate, alkali and chlorite soluble materials and composed of xylose (10.2–12.3 mol%), uronic acid (18.4–22.6 mol%), rhamnose (23.8–26.3 mol%) and sulfate (38.4–41.9 mol%) (Fig. 1, AF4, S1F4, S4F4, NCF3, S*4F3; Tables 2–6). Their chemical composition was close to that of ulvan from *Ulva* collected from "green-tides" [6] and to that of the water-soluble sulfated polysaccharides from *U. lactuca* [15,18]. However, they differed in their elution behaviour from the anion exchanger; Percival and Wold [15] obtained several fractions of similar composition but of different molecular weights by DEAE-cellulose chromatography of the sulfated glucuronorhamnoxyloglycan from *U. lactuca*. In this study, only one minor fraction richer in sulfate (S4F5) was eluted from the anion-exchanger with 1 M NaCl. The partially desulfated and uronic acid-reduced water soluble sulfated polysaccharides from *U. lactuca* were reported [16] to contain 1,4-, 1,3,4-linked rhamnose and xylose, 1,3-linked xylose, 1,2,3-linked rhamnose, 1,3,6-, 1,4- and 1,3-linked glucose (reduced glucuronic acid) and a structural repeating unit of *O*-L-rhamnopyranosyl-(1 → 4)-*O*-D-xylopyranosyl-(1 → 3)-D-glucopyranose (reduced glucuronic acid). This and other oligosaccharides isolated by Haq and Percival [16] demonstrated that all the different sugars were linked together in the sulfated glucuronorhamnoxyloglycan of *U. lactuca*. On the basis of oxidation, methylation and IR analyses, sulfate was localized on O-2 of rhamnose and partially on O-2 of xylose [15]. Methylation analysis of native and chemically modified fractions A, S1F4 and S4F4 isolated from *U. "rigida"* demonstrated that they consisted of 1,4- and 1,2,4-linked rhamnose 3-sulfate, 1,4- and terminal glucuronic acid and 1,4-linked xylose partially sulfated on O-2 in similar proportions for all three fractions studied [26]. These fractions gave similar IR sulfate absorbances [26] to those described by Percival and Wold [15] for the sulfated polysaccharide from *U. lactuca*. From the anion-exchange chromato-

graphic homogeneity, similarities in charge density and their close chemical composition with water-soluble sulfated polysacharides described in the literature from related algal species, these acidic polysaccharidic fractions are considered to belong to the family of sulfated glucuronorhamnoxyloglycan referred to as ulvan for simplicity. The ulvan fractions recovered from the different extracts probably differed in their degree of association with other cell-wall polymers.

A second category of polysaccharides, solubilized only by strong alkali and sodium chlorite from *U. "rigida"*, consisted of polymers rich in uronic acid recovered in the S4F3, NCF2 and S*4F2 fractions from the anion exchange chromatography of the 4 M KOH and chlorite-soluble extracts. In a previous report [25], a 4 M KOH-soluble was identified by ^{13}C NMR spectroscopy from the cell wall of *U. lactuca*. The ^{13}C NMR spectrum of the S4F3 fraction was that of β-1,4-glucurono-oligosaccharides with unsaturated non-reducing ends [27]. Such oligosaccharides attested for the presence of β-1,4-glucuronan in the cell wall of *U. "rigida"* that were most probably degraded by β-elimination reactions during their extraction by 4 M KOH.

The third type of polysaccharides consisted of neutral polymers rich in glucose and xylose recovered from the 1 and 4 M KOH and chlorite extracts (S1aq., S4aq., NCaq., S*4aq.). ^1H NMR and methylation analyses of S1aq. and S4aq. [27] indicated that these sugars were β (1,4)-linked and occured on the same chain, because these polymers were soluble in D_2O and gave five anomeric doublets and no anomeric signal for reducing ends on their ^1H NMR. These glucoxylans may be related to the linear xyloglucans described in the insoluble α-cellulose of *U. lactuca* [22] and because the Xyl:Glc molar ratio of these extracts varied (1.3, 1.1, 1.9 and 0.2 for S1aq., S4aq., NCaq. and S*4aq., respectively) they may form a continuum of molecules with different sequences of glucose and xylose. The latter may affect their ability to self-associate or associate with cellulose through H-bonds, resulting in their different solubilities. The neutral fraction eluted from DEAE fractionation of the oxalate soluble extract (A) also contained glucose and xylose but because the sample was not treated with amyloglucosidase, the starch origin of the glucose cannot be ruled out.

Thus, one water-soluble (ulvan) and two main hemicellulosic (glucuronan and glucoxylan) polysaccharides are found in *U.* "rigida" cell walls. These polysaccharides were incompletely extracted with one solvent condition and, as previously reported [20–22], pure cellulose residues were not obtained, but rather α-cellulose enriched in glucose and xylose. The two-cell thickness of the *Ulva* blade precludes the inaccessibility of the solvents to the polysaccharides as an explaination for their extraction behaviour. Instead, these polymers were probably closely associated by alkali and/or chlorite-labile bonds. Minor protein rich acidic fractions containing glucose, mannose and/or xylose were recovered in the AF1, S1F1, S4F1, NCF1 and S*4F1 fractions from the anion-exchange chromatography of A, S1, S4, NC and S*4F1 extracts, respectively (Tables 1–6). The origin (cell wall, intracellular and/or cuticular) of proteins and their role in the solubility behaviour of the polysaccharides are unknown. Of particular interest is the solubility behaviour of the β (1,4)-D-glucuronan in strong alkali, which has been isolated as water-soluble exopolysaccharides from moulds [34] and bacteria [35]. Future studies will determine whether proteins and charged polysaccharides

(glucoxylans, glucans, mannans, polyuronan) form cell-wall glycoproteins or are co-extracted/eluted as separate entities.

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