ELSEVIER

Carbohydrate Research 304 (1997) 325–333

CARBOHYDRATE RESEARCH

Fine chemical structure analysis of oligosaccharides produced by an ulvan-lyase degradation of the water-soluble cell-wall polysaccharides from *Ulva* sp. (Ulvales, Chlorophyta)

Marc Lahaye *, Magali Brunel, Estelle Bonnin

Institut National de la Recherche Agronomique, Laboratoire de Biochimie et Technologie des Glucides, B.P. 71627, F-44316 Nantes, France

Received 2 June 1997; accepted 22 August 1997

Abstract

A marine bacterium degrading the water-soluble cell wall polysaccharides from *Ulva* sp. (ulvan) has been isolated. The good correlation between ulvan degradation monitored by reducing-power, UV absorbance and viscosimetry, indicated that the crude enzymatic extract contains essentially an *endo*-ulvan lyase activity. This activity was rapidly inhibited by the reaction products which consisted of a series of ulvanobiouronic acid A 3-sulfate [→4)-β-D-Glc*p*A-(1→4)-α-L-Rha*p* 3-sulfate-(1→]*n* with 4-deoxy-L-*threo*-hex-4-enopyranosiduronic acid at the non-reducing end. Other deviant repeating structures with β-D-Xyl*p* or α-L-Ido*p*A replacing β-D-Glc*p*A in the repeating ulvanobiouronic acid disaccharide and the presence of two consecutive (1→4) linked β-D-Glc*p*A demonstrated the great variability and complexity of ulvan chemical structure.

Keywords: Ulvan; *Ulva* sp.; Lyase; NMR; Oligosaccharide

1. Introduction

The marine green seaweeds *Ulva* sp. (Ulvales, Chlorophyta) represent an important biomass that is still little used. Some of them are consumed as sea-lettuce [1] and are rich in dietary fibres [2]. These indigestible polysaccharides are composed of a soft gel forming water-soluble ulvan essentially constituted of D-glucuronic acid, L-iduronic acid, D-xylose, L-rhamnose and sulfate [2–6], alkali-soluble (1→4)-β-D-glucoxylan and (1→4)-β-D-glucuronan [7], and amorphous cellulose containing xylose residues [8,9]. The major repeating disaccharide units in ulvan from sea-lettuce are the aldobiouronic acids [→4)-β-D-Glc*p*A-(1→4)-α-L-Rha*p* 3-sulfate (1→]*n* and [→4)-α-L-Ido*p*A-(1→4)-α-L-Rha*p* 3-sulfate (1→]*n* that have been named ulvanobiouronic acid 3-sulfate A and B, respectively [10]. These particular chemical structures are responsible for the resistance of this water-soluble dietary fibre to *in vitro* fermentations by human faecal flora [11,12] and consequently, for the low degradation of the algal α-cellulose by the

* Corresponding author: E-mail: lahaye@nantes.inra.fr.

0008-6215/97/$17.00
PII S0008-6215(97)00270-X

colonic bacteria [13]. As no information is available in the literature on the enzymatic degradation of ulvan, the aim of this work was to obtain a marine bacterium able to produce enzymes degrading this polysaccharide, to identify the nature of the enzymes and the reaction products. The latter were particularly useful for the fine chemical structure characterization of ulvan.

2. Materials and methods

Materials.—Bacteria were isolated from muds containing decomposing *Ulva* sp. collected on January 1996 at a site of 'green-tide' (Saint-Brieuc bay, Brittany, France). Ulvan was extracted from commercial 'sea-lettuce' (*Ulva* sp.) dry flakes (Nature–Algues, Pleubian) as described [10]. The ash content (500 °C, 18 h followed by 2 h, 900 °C) was 18.4%. The composition of ulvan (g per 100 g of ulvan) was sulfate 21.5, Rha: 20.8, GlcA: 16.0, IdoA: 3.7, Xyl: 3.5, Glc: 2.8 and Gal: 0.7 [6].

Ulvan from 'green-tide' *Ulva* and from *Enteromorpha compressa* were from the laboratory collection [2,4].

Bacteria and enzyme isolations.—Bacteria from mud and isolated bacteria were grown in a liquid broth (pH 7.2) composed per liter of deionized-water: NaCl (22 g), Na_2SO_4 (3.7 g), potassium chloride (0.6 g), potassium bromide (0.1 g), magnesium chloride hexahydrate (10 g), calcium chloride dihydrate (2.94 g), $NaHCO_3$ (0.16 g), sodium hydrogen phosphate dodecahydrate (5 mg), sodium nitrate (25 mg), Difco–Bacto Casamino-acids (ref. A5023002, OSI, Paris, France, 2.5 g), ulvan (2 g) and *Ulva* sp. flakes (2 g). Colonies were isolated from solid medium made of the same salts and Casamino-acid soln to which ulvan (20 g L^{-1}) and agar (15 g L^{-1}, Difco–Bacto, ref. A5014001, OSI, Paris, France) were added. Liquid and solid cultures were incubated at 25 °C. Liquid culture was carried out with agitation in Erlenmeyer flasks with a volume ratio of flask to broth of 5. Gram staining was done using the Gram Color Kit provided by Merck (refs. 1.11885.0001 and 1.10218.0500, Laboratoires Merck–Clévenot, Nogent/Marne, France).

The enzymatic activity was recovered after centrifugation of a 72-h liquid culture (30,000 × g, 30 min, 4 °C) and proteins were precipitated by ammonium sulfate to 80% saturation. The pellet, recovered after 3 h agitation at 4 °C and centrifugation as above, was redissolved in a minimal amount of Tris–HCl buffer (0.1 mol L^{-1}, pH 7.0, 0.025 mol L^{-1} $CaCl_2$) and kept at 4 °C. For some applications, the resolubilized crude proteins were dialyzed extensively against Tris–HCl buffer (0.050 mol L^{-1}, pH 9.0, 0.025 mol L^{-1} $CaCl_2$).

Enzymatic activity assays.—Three different methods were used to determine the enzymatic activity of the liquid bacterial cultures and protein precipitates: reducing-sugar assay, UV absorption at 233 nm and viscosimetry. The reducing-sugar assay was performed according to the Nelson–Somogyi method [14]. The enzymatic reaction was performed as follows: to ulvan soln (100 μL, 27 g L^{-1}, Tris–HCl 0.1 mol L^{-1}, pH 9.0) was added culture supernatant (7000 g, 10 min, 100 μL) or precipitated proteins in Tris–HCl buffer (50 μL) diluted with 50 μL of the same buffer. The mixture was incubated for 1 h at 40 °C. Controls were run with heat-inhibited enzymatic activities (100 °C, 10 min). The reducing-power was measured from 100 μL of the reaction mixture and diluted to 1 mL with deionized-water. The reducing-power obtained was compared to a rhamnose standard curve and the enzymatic activity was expressed in nkat which corresponds to the amount of enzyme necessary to produce nanomoles of reducing rhamnose per second of reaction. The enzymatic reaction for the UV assay was performed using the above conditions. An aliquot of the reaction mixture (100 μL) was diluted to 1 mL with deionized-water prior to observation at 233 nm. The variation of the specific viscosity of ulvan (2 mL of ulvan at 27 g L^{-1} in Tris–HCl 0.1 mol L^{-1}, pH 9.0) containing precipitated protein in buffer (100 μL) was recorded at 40 °C over time using an Ostwald capillary on a SEMATech Viscologic TI 1 viscosimeter (France). The specific viscosity corresponded to the time taken by the reaction soln to pass between two optical detectors in the capillary (T_S) minus the time taken by the buffer containing the enzyme (T_0) and divided by T_0.

$$(T_S - T_0)/T_0$$

Protein content determination.—Proteins in the resolubilized protein precipitate were quantified at 280 nm by comparison with a standard curve obtained with bovine serum albumin.

Gel permeation chromatography.—Ulvan enzymatic degradation products were freeze-dried and redissolved in a minimal amount of sodium nitrate (0.050 mol L^{-1}). The solns were chromatographed through a column of Bio-Gel P2 and Bio-Gel P4 (97 × 2.6 cm, Bio Rad) eluted by sodium nitrate (0.050 mol L^{-1}, 25–27 ml h^{-1}). Peaks were pooled, concentrated by rotary-evaporation, desalted using a

Sephadex G10 column (100 × 1.6 cm, Pharmacia) eluted with water and freeze-dried. Elutions were monitored by differential refractometry and expressed as K_{av} ($K_{av} = (V_e - V_0)/(V_t - V_0)$ with V_t, V_0 being the total and void volume of the column, and V_e the elution volume of the sample).

Nuclear magnetic resonance spectroscopy.—^{1}H and ^{13}C NMR spectra were recorded at 300 K on a Bruker ARX 400 spectrometer from deuterium exchanged (twice in deuterium oxide, 99.9% D) oligosaccharides in 100% D deuterium oxide (Aldrich). Chemical shifts were calculated from the internal acetone resonance attributed to 2.225 and 31.45 ppm for ^{1}H and ^{13}C resonances, respectively. Non-exchangeable proton and carbon assignments of oligosaccharides and sugar sequences were determined from two-dimensional, COSY90, TOCSY (HOHAHA), NOESY, ^{1}H – ^{13}C HMQC, HMQC-TOCSY and HMBC spectra using the conventional pulse sequences provided by Bruker.

3. Results

Among the bacteria growing in the liquid broth with ulvan and *Ulva* sp. flakes, only one colony isolated on solid medium showed ulvanolytic activity once re-incubated in the ulvan liquid medium. On solid medium, this Gram-negative bacterium formed small orange colonies with smooth edges. Ulvanolytic activity in the liquid medium measured by the reducing power assay was maximum when the bacteria were transferred each 60 to 72 h of incubation. Shorter and longer incubation times prior to transfer led to the loss of the activity. Ulvanolytic activity was maximum after 24 h culture and remained stable up to 72 h of incubation (data not shown).

Most of the enzymatic activity (80% of the initial activity) was retained after 2 months at 4 °C in the presence of calcium chloride in the buffer. About half of the activity (45%) was lost when this salt was absent. The optimum temperature for the activity was

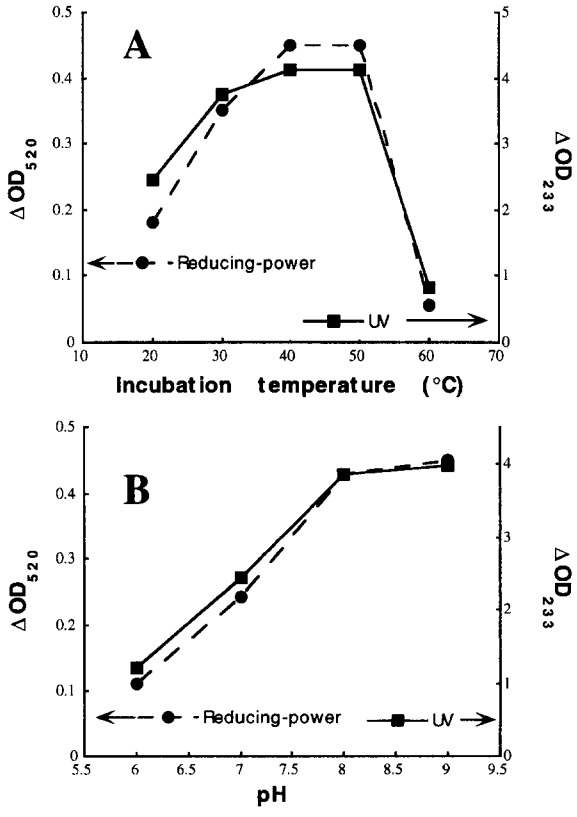

Fig. 1. (A) Effect of temperature and (B) effect of pH on the ulvanolytic activity measured by the reducing power assay (ΔOD_{520}) and by UV absorbtion (ΔOD_{233}).

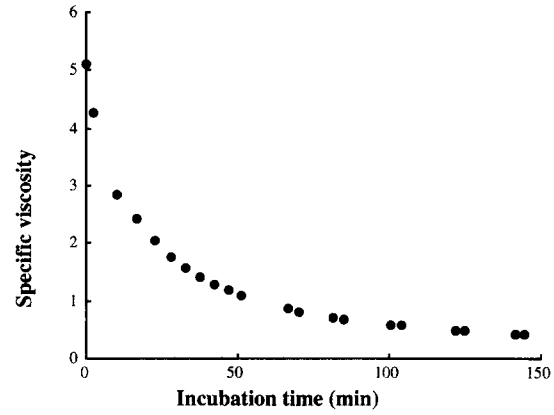

Fig. 2. Evolution of the specific viscosity of ulvan solution (27 g L^{-1} in Tris–HCl 0.1 mol L^{-1}, pH 9.0) in the presence of the ulvanolytic activity (100 μL of 0.8 nkat mL^{-1}) with time.

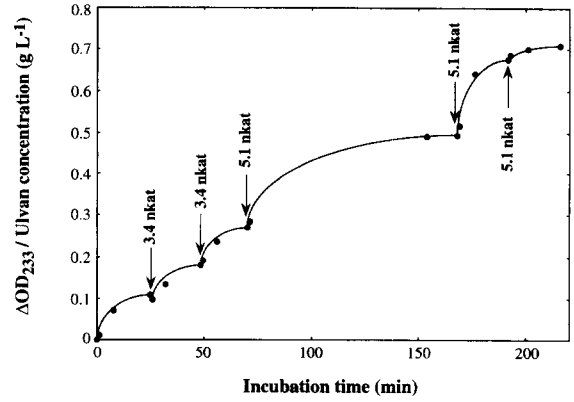

Fig. 3. Kinetics of 'sea-lettuce' ulvan degradation (10 mL of a 4 g L^{-1} solution in Tris–HCl 0.1 mol L^{-1}, pH 9.0, 40 °C) by the ulvan-lyase preparation followed by the increase in UV absorbance at 233 nm.

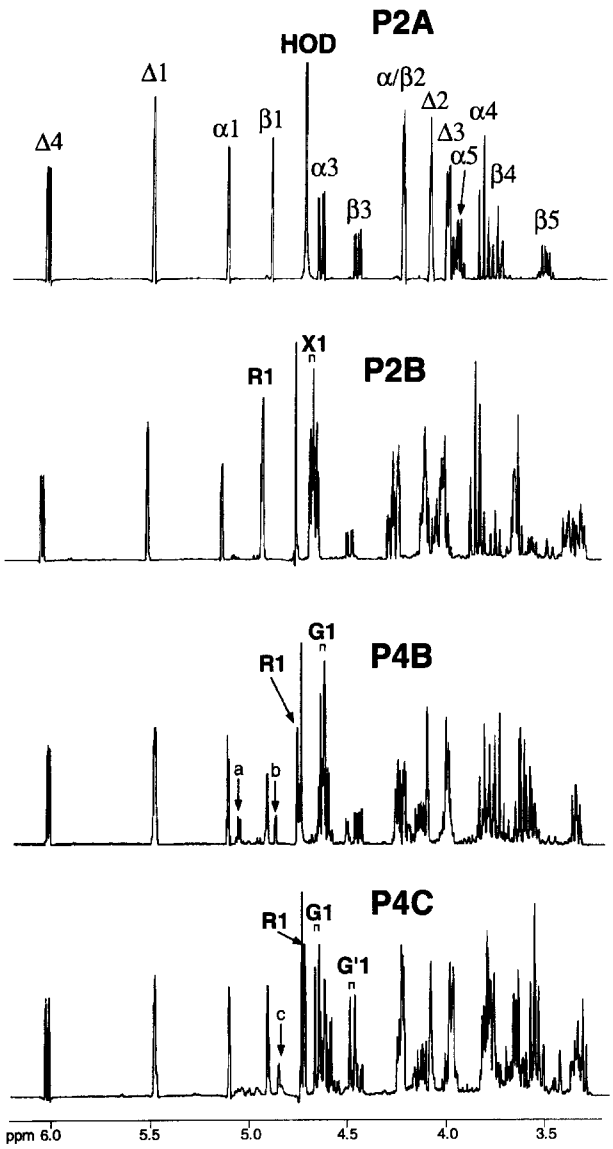

Fig. 4. ^{1}H NMR spectra of oligosaccharides P2A, P2B, P4B and P4C recovered from the ulvan-lyase degraded 'sea-lettuce' ulvan after Bio Gel P2 and P4 chromatography; α, β, Δ, R, X, G, G′ refer to the α- and β-L-Rha*p* 3-sulfate reducing ends, to 4-deoxy-L-*threo*-hex-4-enopyranosiduronic acid at non-reducing end, to α-L-Rha*p* 3-sulfate, to β-D-Xyl*p* and to two different β-D-Glc*p*A residues, respectively; numbers refer to the proton in the different sugars; a and b refer to the anomeric proton of α-L-Ido*p*A and α-L-Rha*p* 3-sulfate linked to α-L-Ido*p*A, respectively; c: unknown.

40 to 50 °C (Fig. 1A) and after 25 h of incubation at 40 °C, 94% of the activity was retained. The optimum pH for the enzymatic degradation was near 9 although higher values were not tested (Fig. 1B). The specific viscosity of an ulvan soln decreased rapidly on action of the enzymes (Fig. 2). Good correlations were observed between the activity measured by reducing-power and UV absorbance ($r^2 = 0.957$), and reducing-power and viscosimetry ($r^2 = 0.949$). The specific activity of the crude preparation was 8.1 nkat

Table 1
^{1}H and ^{13}C NMR chemical shifts of ulvan oligosaccharides obtained after enzymatic degradation and gel permeation chromatography; α, β, Δ, R, X, G, G′ refer to the α- and β-Rha*p* 3-sulfate reducing ends, to 4-deoxy-L-*threo*-hex-4-enopyranosidur onic acid at the non-reducing end, to α-L-Rha*p* 3-sulfate, to β-D-Xyl*p* and to two different β-D-Glc*p*A residues, respectively; numbers refer to the proton and carbon in the different sugars

		1	2	3	4	5	5′	6
P2A								
α	^{1}H	5.12	4.23	4.65	3.82	3.95		1.16
	^{13}C	94.85	70.61	79.94	77.50	68.08		17.99
β	^{1}H	4.90	4.23	4.47	3.76	3.51		1.18
	^{13}C	94.25	70.98	81.77	77.18	71.43		17.99
Δ	^{1}H	5.49	4.09	4.01	6.02	–		–
	^{13}C	100.28	69.10	65.25	107.62	145.33		170.43
P2B								
α	^{1}H	5.11	4.25	4.65	3.81	3.99	–	1.31
	^{13}C	94.63	70.49	79.46	78.55	68.68	–	18.34
β	^{1}H	4.92	4.27	4.47	3.73	3.55	–	1.32
	^{13}C	94.21	70.93	81.43	77.99	71.85	–	18.34
X	^{1}H	4.66	3.30	~3.63	~3.65	3.37	4.09	
	^{13}C	104.75	74.51	74.80	75.24	63.56		
R	^{1}H	4.91	4.22	4.64	3.84	4.03	–	1.18
	^{13}C	98.70	70.13	80.01	77.47	68.62	–	17.81
Δ	^{1}H	5.49	4.09	4.00	6.02	–	–	–
	^{13}C	100.34	69.12	65.33	107.53	145.49	–	170.51
P4B								
α	^{1}H	5.11	4.25	4.63	3.81	4.00		1.25
	^{13}C	94.62	70.53	79.26	79.47	68.90		18.45
β	^{1}H	4.91	4.26	4.45	3.71	3.54		1.35
	^{13}C	94.19	70.95	81.21	79.02	72.11		18.45
G	^{1}H	4.63	3.34	3.63	3.57	3.75		–
	^{13}C	104.36	74.93	75.05	80.06	77.17		176.48
R	^{1}H	4.76	4.22	4.62	3.81	4.14		1.14
	^{13}C	101.21	70.16	79.98	77.54	68.60		17.66
Δ	^{1}H	5.49	4.10	3.99	6.03	–		–
	^{13}C	100.38	69.09	65.32	107.75	145.30		170.35
P4C								
α	^{1}H	5.11	4.24	4.64	3.79	4.00		1.33
	^{13}C	64.12	70.52	79.28	79.41	68.88		18.45
β	^{1}H	4.91	4.26	4.46	3.72	3.55		1.34
	^{13}C	94.19	70.95	81.22	78.96	72.09		18.45
G	^{1}H	4.67	3.36	~3.66	3.67	3.82		–
	^{13}C	104.23	74.31	75.34	82.01	76.91		176.24
G′	^{1}H	4.49	3.33	3.58	3.55	3.79		–
	^{13}C	103.46	74.55	74.98	79.85	76.91		176.24
R	^{1}H	4.73	4.23	4.61	3.80	4.14		1.14
	^{13}C	101.22	70.09	79.98	77.54	68.63		17.65
Δ	^{1}H	5.48	4.10	3.99	6.02	–		–
	^{13}C	100.38	69.09	65.34	107.60	145.44		170.44

mg^{-1} according to the reducing power assay. Using this preparation, the final degradation of ulvan from 'green-tide' *Ulva* and *E. compressa* were 69 and 13%, respectively, of that measured with ulvan from edible sea-lettuce.

Sea-lettuce ulvan degradation by the precipitated and dialyzed proteins was slow and rapidly reached a plateau (Fig. 3). Further degradation of ulvan was achieved by repeated additions of enzymes until no more reducing sugar was produced. At that stage, the sugar released corresponded to 7.1% of their initial content in ulvan on the basis of reducing-power.

The degradation products from the latter experiment were chromatographed through Bio-Gel P2 and two major peaks eluting at K_{av} 0.41 and 0.23 were collected (referred to as P2A and P2B, respectively). The material eluting near the void volume (K_{av} 0–0.18) of the column were re-chromatographed through Bio-Gel P4 and two major peaks eluting at K_{av} 0.35 and 0.23 and a small shoulder at K_{av} 0.41 were collected and were referred to as P4B, P4C and P4A, respectively. The material eluting between K_{av} 0–0.11 was not studied further.

These different fractions were identified by NMR spectroscopy. The ^{1}H NMR resonances of fraction P2A (Fig. 4) were all unambiguously assigned (Table 1) from a COSY experiment and by comparison with NMR data for ulvanobiouronic acid 3-sulfate A [15] and (1 → 4) linked β-D-glucuronan oligomers having 4-deoxy-L-*threo*-hex-4-enopyranosiduronic acid at the non-reducing end [16]. The ^{13}C NMR chemical shifts were obtained from an HMQC experiment (Table 1). According to the integral values of the anomeric protons and to a NOESY experiment, P2A was identified as a disaccharide composed of non-reducing 4-deoxy-L-*threo*-hex-4-enopyranosiduronic acid linked to O-4 of L-Rha*p* 3-sulfate at the reducing end (Fig. 5). The ^{1}H NMR resonances of Fraction P2B (Fig. 4) were assigned (Table 1) from the COSY experiment and by comparison with the resonances of P2A and of D-Xyl containing ulvan oligosaccharides [15]. The ^{13}C NMR chemical shifts were obtained

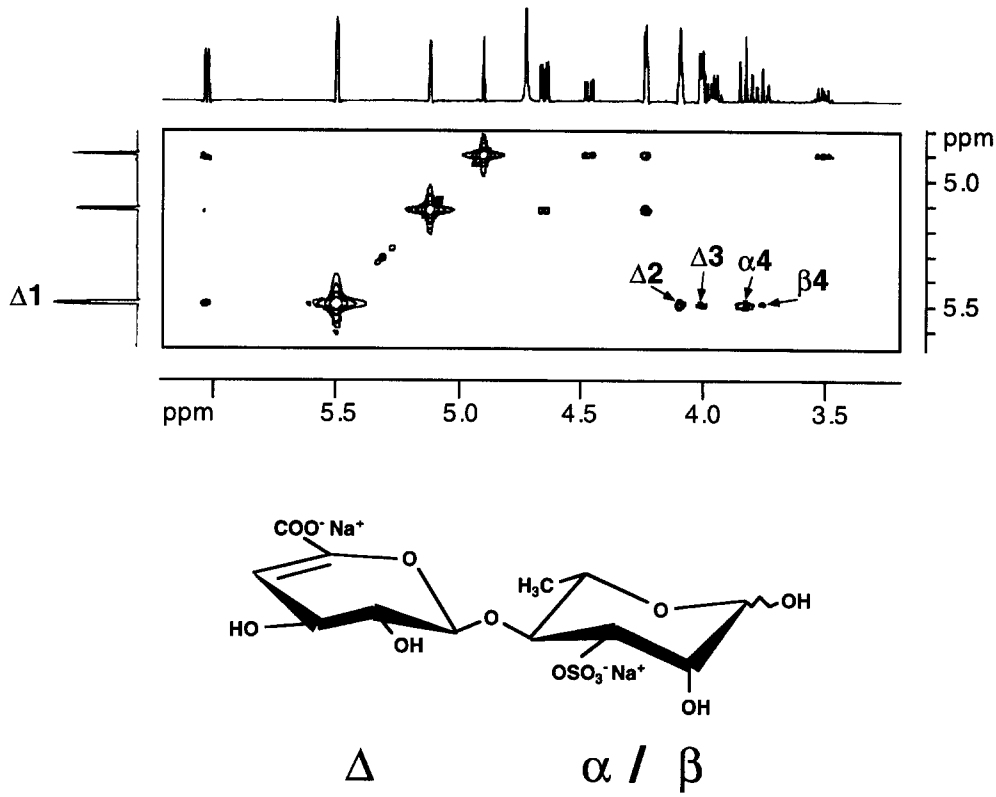

Fig. 5. Partial NOESY map and chemical structure of oligosaccharide P2A; Δ, α and β refer to protons in the 4-deoxy-L-*threo*-hex-4-enopyranosiduronic acid and in the α- and β-L-Rha*p* 3-sulfate at the reducing end, respectively; 1024 experiments of 2K data points and 8 transients each with a recycling time of 1.4 s were recorded and transformed on 2 × 2K data points with unshifted sine bell multiplication in both dimensions. The mixing time was 500 ms.

from a HMQC experiment and according to the intensity of the anomeric carbons, P2B was composed of four sugars: one 4-deoxy-L-*threo*-hex-4-enopyranosiduronic acid at the non-reducing end, one internal α-L-Rha*p* 3-sulfate and D-Xyl*p* residues and one L-Rha*p* 3-sulfate at the reducing-end. All the sugars (except the non-reducing end) were linked through O-4 as demonstrated by the HMBC experiment (Fig. 6).

According to the ^{1}H NMR spectrum, Fraction P4A was the same oligosaccharide as P2B (data not shown). The major peak P4B yielded ^{1}H NMR resonances (Fig. 4) that were attributed from a COSY experiment and by comparison with the data obtained from P2A and from ulvanobiouronic acid 3-sulfate A (Table 1) [15]. It was composed of one 4-deoxy-L-*threo*-hex-4-enopyranosiduronic acid at the non-reducing end, one internal β-D-Glc*p*A and α-L-Rha*p* 3-sulfate and one reducing-end L-Rha*p* 3-sulfate. The ^{13}C NMR signals were readily attributed from an HMQC experiment (Table 1) and, according to the intensity of the representative anomeric carbons of the different sugar residues, this oligosaccharide was identified as a tetramer. The HMBC experiment demonstrated only (1 → 4) linkages with the sugar sequence in Fig. 7. Minor signals on the ^{1}H NMR spectrum of P4B (a and b on Figs. 4 and 7) were attributed to minor amounts of α-L-Ido*p*A (a: H-1 and H-5 of α-L-Ido*p*A at 5.066 and 4.517 ppm, respectively and b: H-1 of α-L-Rha*p* 3-sulfate linked to α-L-Ido*p*A 4.880 ppm) replacing for the internal β-D-Glc*p*A residue [10].

The P4C ^{1}H NMR spectrum (Fig. 4) was assigned from a COSY experiment and by comparison with the data obtained for P4B (Table 1). The ^{13}C NMR spectrum was assigned from an HMQC experiment (Table 1) and according to the anomeric carbon intensities, this oligosaccharide was composed of five

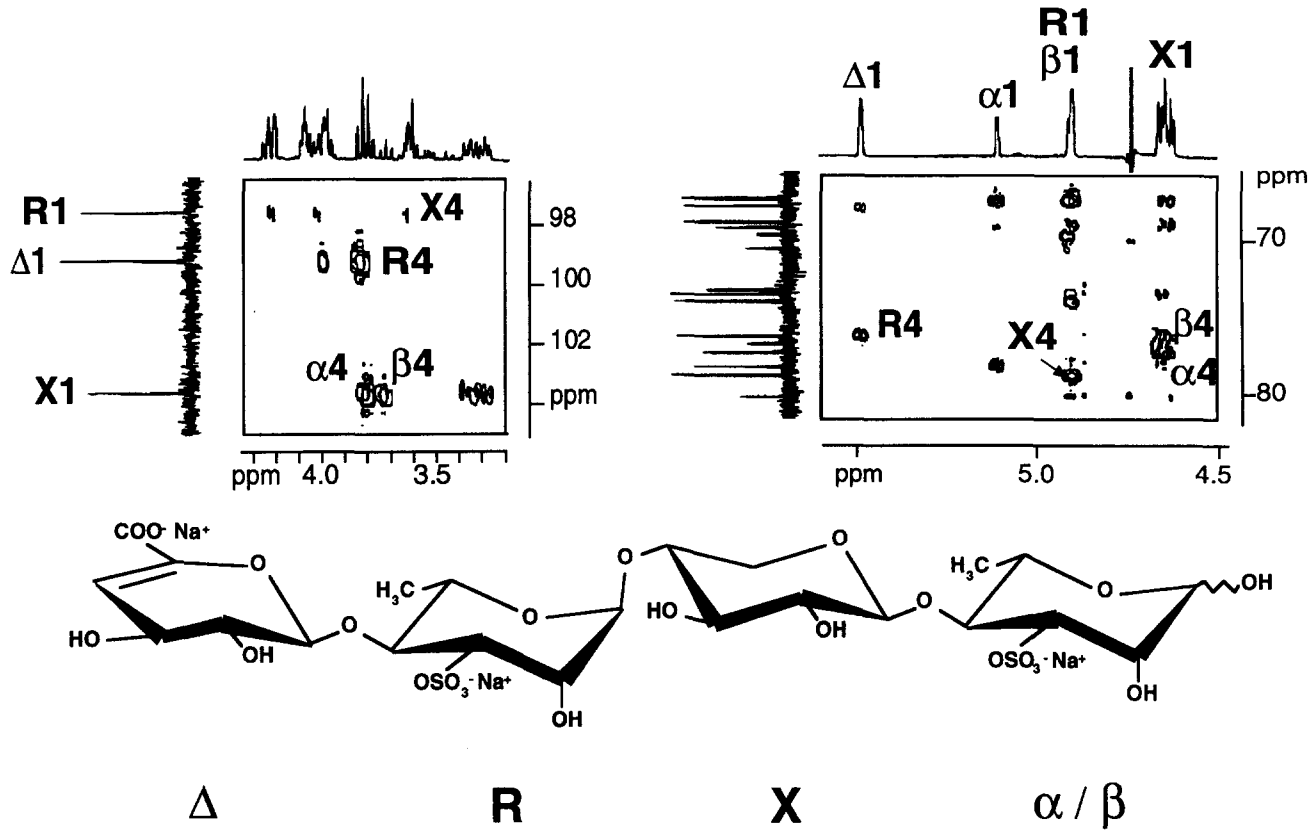

Fig. 6. Partial HMBC maps and chemical structure of oligosaccharide P2B; Δ, R, X, α and β refer to protons in 4-deoxy-L-*threo*-hex-4-enopyranosiduronic acid, α-L-Rha*p* 3-sulfate, β-D-Xyl*p*, and in the α- and β-L-Rha*p* 3-sulfate at the reducing end, respectively; 512 experiments of 2K data points and 64 transients each with a recycling time of 1.4 s were recorded and transformed on 1 × 2K data points with a $\pi/4$ shifted sine bell multiplication in the F2 dimension and an exponential multiplication (line broadening of 0.1 Hz) in the F1 dimension. The delay for the long range coupling evolution was 100 ms.

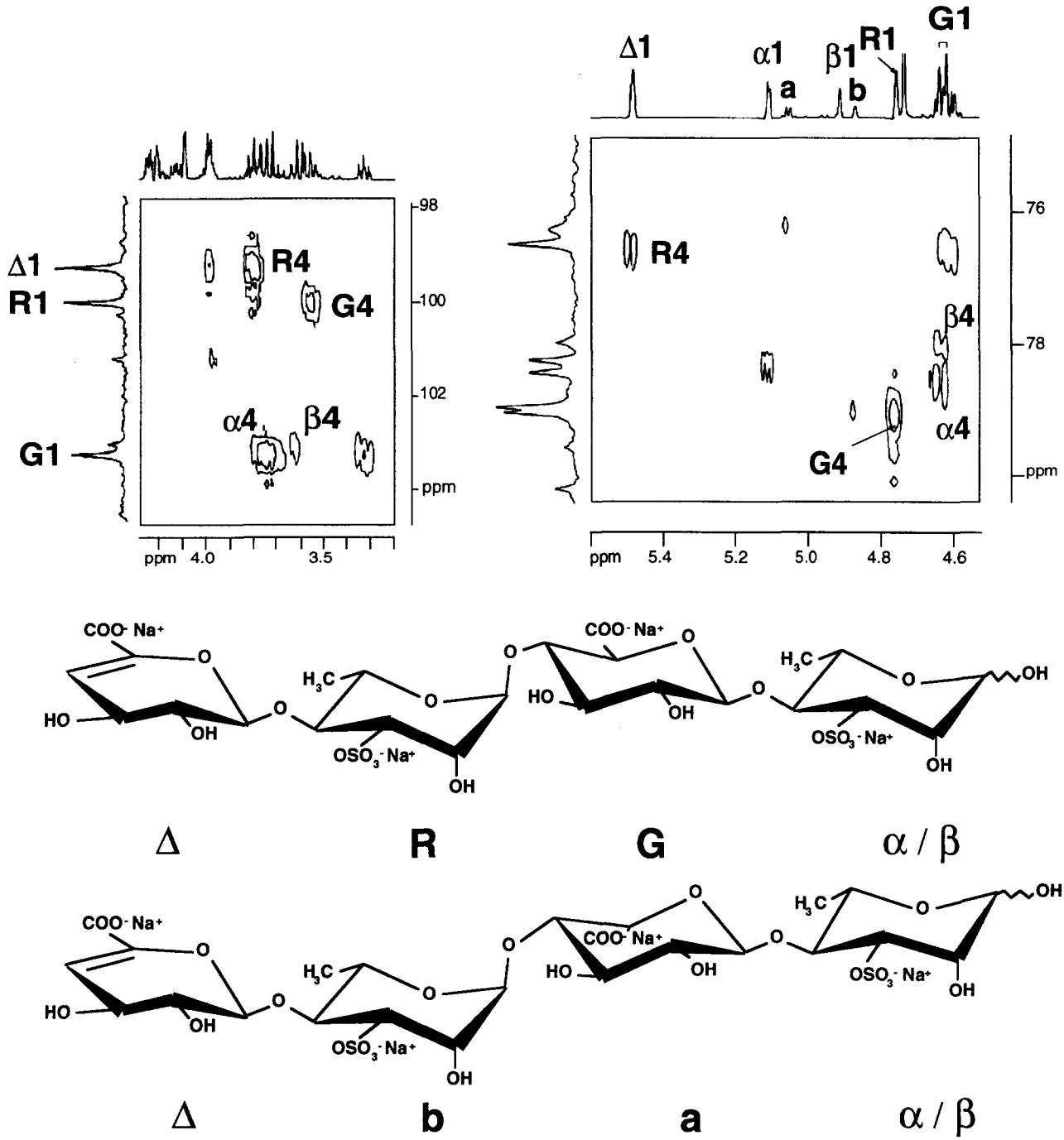

Fig. 7. Partial HMBC maps and chemical structure of oligosaccharide P4B; Δ, R, G, α and β refer to protons in 4-deoxy-L-*threo*-hex-4-enopyranosiduronic acid, α-L-Rha*p* 3-sulfate, β-D-Glc*p*A, and in the α- and β-L-Rha*p* 3-sulfate at the reducing end, respectively; a and b refer to the anomeric protons of α-L-Ido*p*A and α-L-Rha*p* 3-sulfate linked to α-L-Ido*p*A, respectively; conditions are as in Fig. 6.

sugar residues: one 4-deoxy-L-*threo*-hex-4-enopyranosiduronic acid at the non-reducing end, one internal α-L-Rha*p* 3-sulfate and one reducing L-Rha*p* 3-sulfate and two β-D-Glc*p*A residues. The sequence of these different sugars was determined by the HMBC experiment (Fig. 8). The correlation peaks observed demonstrated that the non-reducing end (Δ) was linked to O-4 of an internal α-L-Rha*p* 3-sulfate residue (R) which was itself linked to O-4 of β-D-Glc*p*A (G′). This uronic acid (G′) was linked to O-4

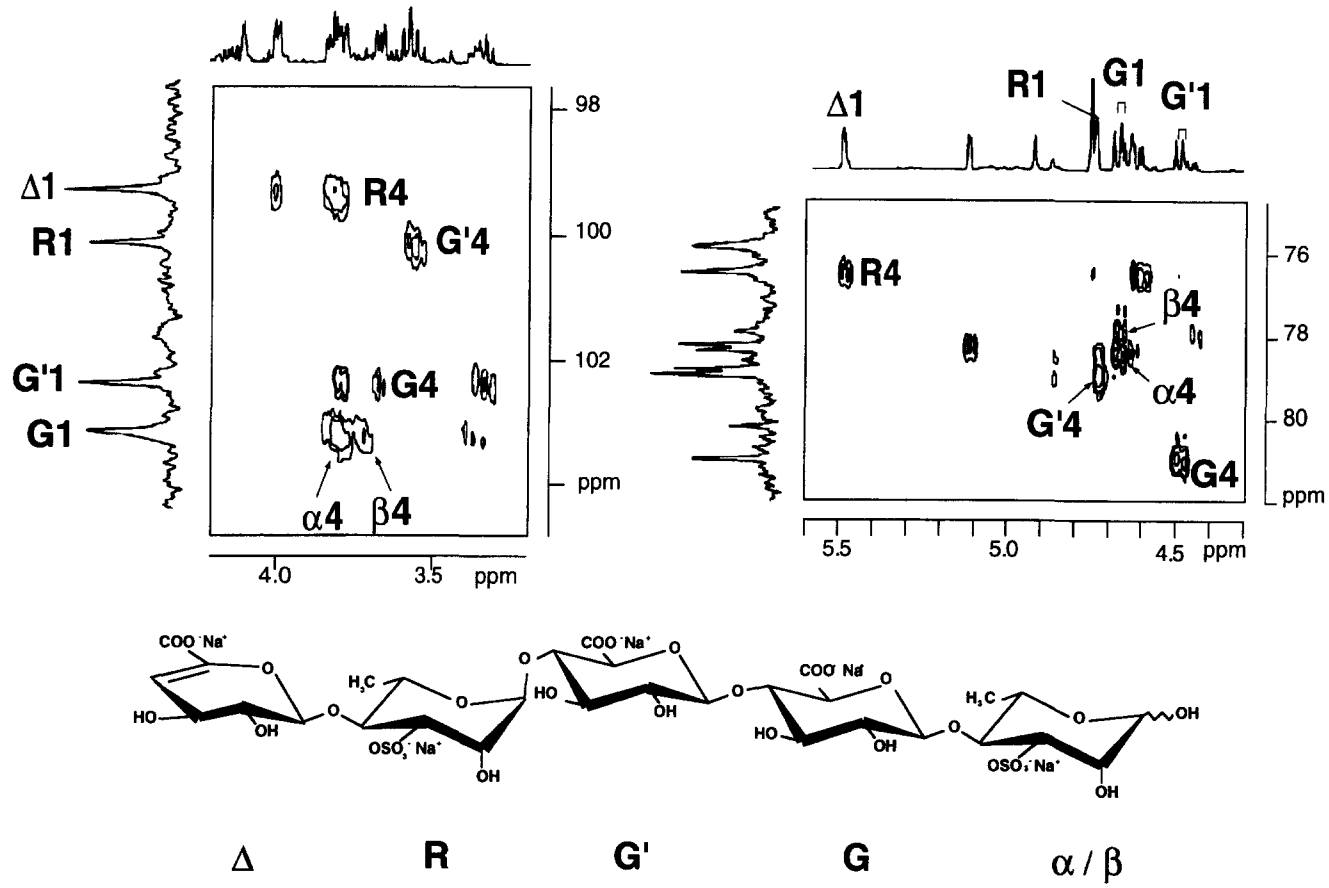

Fig. 8. Partial HMBC maps and chemical structure of oligosaccharide P4C; Δ, R, G, α and β refer to protons in 4-deoxy-L-*threo*-hex-4-enopyranosiduronic acid, α-L-Rha*p* 3-sulfate, β-D-Glc*p*A, and in the α- and β-L-Rha*p* 3-sulfate at the reducing end, respectively; the (') distinguishes between the two β-D-Glc*p*A residues; conditions are as in Fig. 6.

of another β-D-Glc*p*A (G) itself linked to the reducing end L-Rha*p* 3-sulfate (α/β) (Fig. 8).

4. Discussion

Few families of degrading enzymes have been described acting on algal matricial polysaccharides. The main documented ones are alginate lyases (mannuronate and guluronate lyases) and galactan hydrolases (β- and α-agarases, κ- and ι-carrageenases) which were essentially searched for the study of the chemical structure of their substrates and for the production of algal protoplasts [17–22]. The present report is the first description of an enzyme preparation degrading the water-soluble polysaccharides from *Ulva* sp. The identification of 4-deoxy-L-*threo*-hex-4-enopyranosiduronic acid residue at the non-reducing end of the reaction products by the UV absorbance, and by NMR spectroscopy, unambiguously established the nature of the activity as a lyase. The rapid decrease in viscosity of ulvan soln during its degradation by the lyase indicated that the depolymerization was *endo*-molecular. The good correlation between production of reducing-power, appearance of UV absorption and decrease in viscosity also indicated that this endo-lyase was the main acting enzyme in the crude protein extract. Ulvan degradation kinetics demonstrated that the activity was rapidly stopped. This inhibition was probably not due to the denaturation of the lyase as the activity was stable for 25 h in the buffer condition used. Instead, the reaction products more likely inhibited the enzyme preparation as further additions of lyase aliquots allowed the degradation to proceed until all the available sites on ulvan chains have been cleaved. The chemical structure of the unsaturated oligosaccharides produced, which is based on the repetition of the ulvanobiouronic acid 3-sulfate A (dp 2 and dp 4), confirmed the sulfated aldobiouronic acid as the main building block in the ulvan backbone [10]. However, deviant structures with β-D-Xyl*p* or α-L-Ido*p*A replacing β-D-Glc*p*A in the repeating ulvanobiouronic acid 3-sulfate A are also present together with an

irregular distribution of β-D-Glc*p*A which occurs as two consecutive 4-linked residues. The presence of α-L-Ido*p*A in ulvanobiouronic acid 3-sulfate B repeating units was in agreement with the identification of such structures in the autohydrolysate of 'sea-lettuce' ulvan [10]. These, or other deviant structures, may be present in higher concentrations or in a different sequences in ulvan from 'green-tide' *Ulva* and from *E. compressa* explaining the lower degradation of these ulvanobiouronic acid containing polysaccharides [6] compared to 'sea-lettuce' ulvan. Different NMR chemical shifts were used to detect the presence of the various repeating structures. The 103–104 ppm carbon signal region of ulvan ^{13}C NMR spectra can hardly be used to fingerprint the polysaccharide structure since the C-1 signals for β-D-Xyl*p* (104.7 ppm), the different β-D-Glc*p*A (103.5, 104.2 ppm) and α-L-Ido*p*A (103.9 ppm [10]) most likely overlap in the polysaccharides spectra. Instead, β-D-Xyl*p* can be suspected by the presence of its C-5 signal at about 63.6 ppm and the C-1 of the α-L-Rha*p* 3-sulfate linked to β-D-Xyl*p* at about 98.7 ppm and, contiguous β-D-Glc*p*A can be detected by the signal of C-4 at about 82.0 ppm for the β-D-Glc*p*A (1 →]*n* sequences.

Acknowledgements

This work was supported in part by the VANAM programme of the Pays de la Loire Region. The authors thank Mlle J. Vigouroux for the excellent technical assistance, Mr. B. Cahagnier and Mr. L. Helary from the INRA–LMTC (Nantes) for their help and the fruitful discussions.

References

[1] S. Mabeau and J. Fleurence, *Trends Food Sci. Technol.*, 4 (1993) 103-107.
[2] M. Lahaye and D. Jegou, *J. Appl. Phycol.*, 5 (1993) 195-200.
[3] E. Percival and R.H. McDowell, *Chemistry and Enzymology of Marine Algal Polysaccharides*, Academic Press, London, 1967, 219 pp.
[4] M. Lahaye and M.A.V. Axelos, *Carbohydr. Polym.*, 22 (1993) 261-265.
[5] B. Ray and M. Lahaye, *Carbohydr. Res.*, 274 (1995) 251-261.
[6] B. Quemener, M. Lahaye, and C. Bobin-Dubigeon, *J. appl. Phycol.*, 9 (1997) 179–188.
[7] M. Lahaye, B. Ray, S. Baumberger, B. Quemener, and M.A.V. Axelos, *Hydrobiologia*, 326/327 (1996) 473-480.
[8] J.A. Cronshaw, A. Myers, and R.D. Preston, *Biochim. Biophys. Acta*, 27 (1958) 89-103.
[9] M. Lahaye, D. Jegou, and A. Buléon, *Carbohydr. Res.*, 262 (1994) 115-125.
[10] M. Lahaye, F. Inizan, and J. Vigouroux, Carbohydr. Polym. (1997) accepted for publication.
[11] C. Bobin-Dubigeon, M. Lahaye, and J.-L. Barry, *J. Sci. Food Agric.*, 73 (1997) 160-168.
[12] M. Durand, P. Beaumatin, B. Bulman, A. Bernalier, J.P. Grivet, M. Serezat, G. Gramet, and M. Lahaye, *Reprod. Nutr. Dev.*, 37 (1997) 267-283.
[13] C. Bobin-Dubigeon, M. Lahaye, F. Guillon, J.-L. Barry, and D.J. Gallant, *J. Sci. Food Agric.*, (1997) in press.
[14] N. Nelson, *J. Biol. Chem.*, 153 (1944) 375-380.
[15] M. Lahaye and B. Ray, *Carbohydr. Res.*, 283 (1996) 161-173.
[16] L. Dantas, J. Courtois, B. Courtois, J.-P. Seguin, C. Gey, and A. Heyraud, *Carbohydr. Res.*, 265 (1994) 303-310.
[17] D.M. Butler, L.V. Evans, and B. Kloareg, in I. Akatsuka (Ed.), *Introduction to Applied Phycology*, SPB Academic Publishing, The Hague, 1990, pp. 647-668.
[18] C. Greer and W. Yaphe, *Bot. Mar.*, 27 (1984) 479-484.
[19] A. Heyraud, C. Gey, C. Leonard, C. Rochas, S. Girond, and B. Kloareg, *Carbohydr. Res.*, 289 (1996) 11-23.
[20] M. Lahaye, W. Yaphe, M.T. Phan Viet, and C. Rochas, *Carbohydr. Res.*, 190 (1989) 249-265.
[21] K. Østgaard, *Hydrobiologia*, 260/261 (1993) 513-520.
[22] C. Rochas, P. Potin, and B. Kloareg, *Carbohydr. Res.*, 253 (1994) 69-77.