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NMR spectroscopy analysis of oligoguluronates and oligomannuronates prepared by acid or enzymatic hydrolysis of homopolymeric blocks of alginic acid. Application to the determination of the substrate specificity of *Haliotis tuberculata* alginate lyase

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Abstract

The ^{1}H and ^{13}C NMR chemical shifts of the various saturated and unsaturated trimers obtained by acid or enzymatic depolymerisation of homopolymeric blocks of alginates are reported. In addition, ^{13}C NMR chemical shifts are assigned for several saturated oligomers of higher polymerisation degrees. Breakdown of alginate and of homopolymeric alginate blocks by *Haliotis tuberculata* alginate lyase was monitored with ^{1}H NMR spectroscopy and the signals relevant to the identification of the lyase products are pointed out. The enzyme performs β-elimination on the mannuronic acid residues, independently of their immediately surrounding neighbours. Application of this approach to the analysis of the substrate specificity of alginate lyases is discussed. © 1996 Elsevier Science Ltd.

Keywords: *Haliotis tuberculata*; Alginate lyase; Substrate specificity; NMR; Alginic acid; Oligomannuronates; Oligoguluronates

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1. Introduction

Alginic acid is a linear polymer composed of (1 → 4)-linked β-D-mannuronopyranosyl and α-L-guluronopyranosyl units arranged both in homo- or hetero-polymeric sequences [1,2]. The proportion and sequential arrangement of the uronic acid residues depend upon the algal species and the type and age of the tissues used for the alginate preparation [3]. As both the biological and physico-chemical properties of alginates depend on their sequences [4,5], numerous studies have been devoted to their structural analysis. In particular, high resolution ^{1}H and ^{13}C NMR spectroscopy markedly improved the sequential analysis of alginates, by allowing to determine the different diad and triad frequencies in the polymer [6–9].

Enzymes that depolymerize alginates have been detected in a variety of bacteria and marine molluscs. They are lyases (EC 4.2.2.3), that display variable specificities for the homo- and hetero-polymeric sequences and cleave a hexose-1,4-α- or β-uronic acid sequence by β-elimination (e.g., [10–19]). Alginate lyases have been used to evaluate the alginate structure [20,21] as well as for the preparation of oligouronates with antiviral, antitumoral or plant growth- and defense-stimulating activities [22–24]. In such applications, it is of interest to precisely identify the glycosidic linkage which is the site of β-elimination. Yet, although ^{1}H and ^{13}C NMR spectroscopy have been used for the analysis of alginate lyase products [12,24,25], no comprehensive assignment of the chemical shifts of oligoalginates is available. In this paper, an extensive ^{1}H and ^{13}C NMR spectroscopy analysis of various saturated and unsaturated oligomannuronates and oligoguluronates obtained by acid or enzymatic treatment of alginates is reported. Based on these data, we now propose a rapid and reliable method for the investigation of the substrate specificity of alginate lyases.

2. Experimental

Substrates.—Sodium alginates for preparation of homopolymeric blocks were kindly provided by Sanofi BioIndustries (S.B.I., Carentan, France) and Sobalg (Landerneau, France). Polyguluronate (G) and polymannuronate (M) homopolymeric blocks were prepared by two cycles of HCl-hydrolysis according to Haug et al. [26]. The purity of the homopolymeric sequences was assessed by ^{1}H NMR analysis. M Blocks contained 5–10% guluronate and G blocks contained about 5% mannuronate monomers. Alginates from *Macrocystis pyrifera* were supplied by Sigma.

Enzymes.—Klebsiella pneumoniae alginate lyase was kindly provided by K. Østgaard [18]. *H. tuberculata* alginate lyase was prepared from abalone freshly collected at Roscoff, Brittany and partially purified by CMC-exchange chromatography as described previously, leading to a partial purification factor of ≃ 100-fold relative to the crude extract [27].

Specificity determination.—Degradation of *Macrocystis pyrifera* sodium alginate and of homopolymeric blocks with *Haliotis tuberculata* alginate lyase was directly performed in the NMR tube, at room temperature with 1 mL of 1% substrate solution in

D_2O, for 15 min with various enzyme concentrations (2–12 U). ^1H NMR Spectra were then recorded at 80 °C.

Preparation and fractionation of oligouronates.—Oligomannuronates and oligoguluronates were obtained by acid hydrolysis of the 0.1% (w/v) corresponding block solution in 10^{-3} N HCl at 100 °C for 5 and 7 h, respectively. 4,5-Unsaturated oligomannuronates (referred to as ΔM) were prepared by incubation for 6 h at room temperature of 0.1% (w/v) block solution with 1 unit mL^{-1} of *H. tuberculata* alginate lyase [27] while unsaturated oligoguluronates (ΔG) were obtained by the degradation of G blocks with 1 unit mL^{-1} of *Klebsiella pneumoniae* alginate lyase. Oligouronates were then size-fractionated by preparative gel filtration chromatography on a 100×2.5 cm Bio-Gel P-6 column, eluted with 5×10^{-2} N $NaNO_3$ solution at a rate of 90 mL/h [28]. Each fraction was desalted by ultrafiltration on YC 05 Diaflo membrane, purified by HPLC on a preparative 250×22 mm Nucleosil SB column (Interchim), desalted again and freeze-dried. The dp of arbitrarily chosen oligosaccharides was confirmed by FABMS.

NMR Spectrometry studies.—NMR analysis of oligoalginates was performed at 45 °C, using an AC-300 Bruker spectrometer with a 5 mm ^1H–^{13}C dual probe. ^1H NMR spectra were obtained using a spectral width of 3000 Hz, a 16K data-block size and a pulse sequence of 8 ms; 16 scans with $AQ = 2.73$ s were accumulated. The H_2O signal was presaturated using the standard Bruker Presat sequence, with a delay of 3 s and a decoupler power of 20 dB at low range. 2D Proton–proton correlation experiments and 1D TOCSY experiments were performed on a Unity 400 Varian instrument, equipped with a reverse 5 mm ^1H-probe. ^{13}C NMR Spectra were recorded using a spectral width of 15000 Hz, a 16K data-block size and a pulse sequence of 6 ms; 2000 scans were accumulated, with standard composite pulse decoupling (C.P.D.) conditions. Carbon assignments were obtained using a HMQC pulse sequence on a Unity 400 Varian spectrometer. Acetone was used as the external standard ($\delta H = 2.10$ ppm, $\delta C = 31.45$ ppm).

3. Results and discussion

NMR Spectroscopy analysis of unsaturated and saturated oligoalginates.—The 300-MHz proton NMR spectra of *O*-(4-deoxy-α-L-*erythro*-hex-4-enopyranosyluronic acid)-(1 → 4)-*O*-(α-L-gulopyranosyluronic acid)-(1 → 4)-*O*-α-L-gulopyranuronic acid (referred to as DGG), *O*-(4-deoxy-α-L-*erythro*-hex-4-enopyranosyluronic acid)-(1 → 4)-*O*-(β-D-mannopyranosyluronic acid)-(1 → 4)-*O*-β-D-mannopyranuronic acid (ΔMM), *O*-(α-L-gulopyranosyluronic acid)-(1 → 4)-*O*-(α-L-gulopyranosyl-uronic acid)-(1 → 4)-*O*-α-L-gulopyranuronic acid (GGG) and of *O*-(β-D-mannopyranosyluronic acid)-(1 → 4)-*O*-(β-D-mannopyranosyluronic acid-(1 → 4)-*O*-β-D-mannopyranuronic acid (MMM) are given in Fig. 1. The chemical shifts of anomeric protons were assigned according to previous data for alginate polymers [9] and oligomers [12,24]. In both unsaturated and saturated oligomannuronates, the peak at 5.09–5.11 ppm is assigned to the resonance of the reducing-end H-1(α). The apparent single peak at 4.78–4.81 ppm, with a small coupling constant (0.9 Hz), corresponds to the axially oriented H-1 signal of the

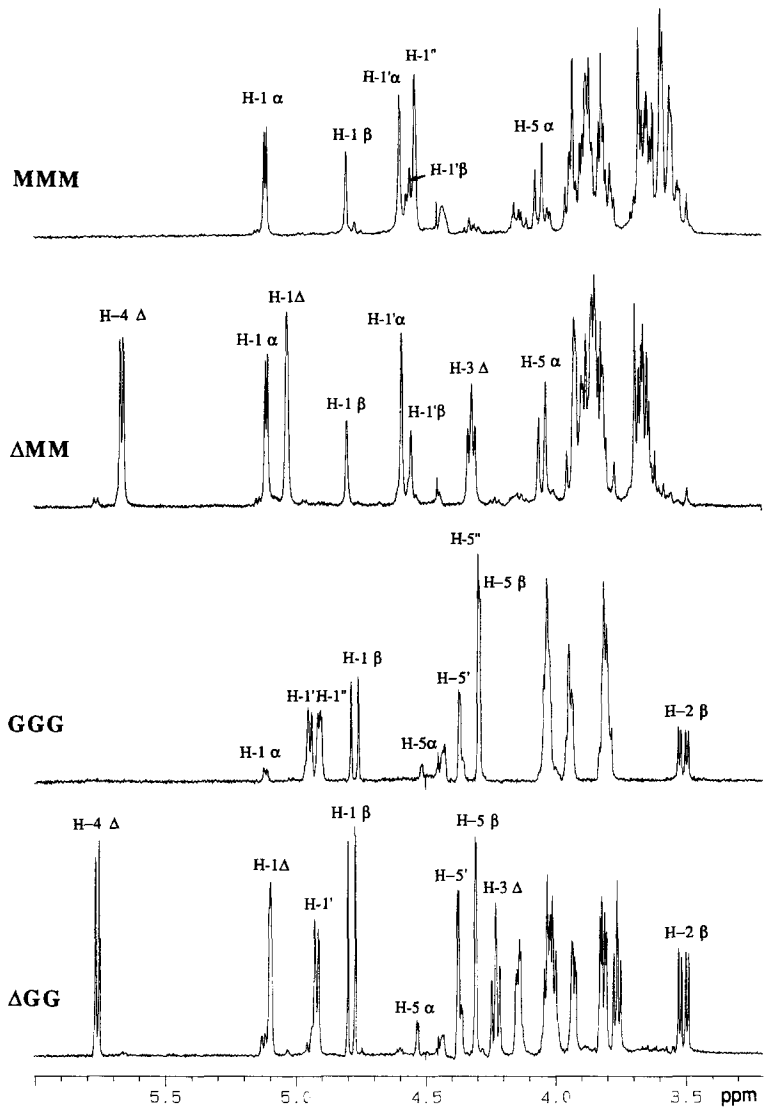

Fig. 1. ^{1}H NMR (300 MHz) spectra of solutions (20 mg mL^{-1}) in D_2O at pD 7 and 45 °C of sodium salts of various trimers obtained by acid or enzymatic hydrolysis of poly-mannuronate and -guluronate alginate blocks. The most relevant signals are pointed out as follows: the prime (′) indicates the residue closest to the reducing end; (″) refers to the non-reducing end glycosyluronic unit; (Δ) refers to the unsaturated non-reducing end.

β-anomer. Two signals are seen for the H-1 protons of the central, intra-chain residue, at 4.53 and at 4.57 ppm for ΔMM, and 4.57–4.60 ppm for MMM, depending on the anomeric form of the reducing end proton, β or α, respectively. Regarding the H-1 protons at the non-reducing ends, signals at 4.56 and 5.01 ppm were observed for the saturated and unsaturated forms, respectively. Based on these data, the complete ^{1}H

Table 1
^{1}H NMR chemical shifts of oligoalginate trimers obtained by acid and enzymatic hydrolysis of poly-mannuronate and -guluronate blocks

	Chemical shifts (ppm) [a]				
	H-1	H-2	H-3	H-4	H-5
MMM					
α	5.11	3.82	3.88	3.92	4.06
β	4.81	3.87	3.67	3.81	3.70
(α')	4.60	3.89	3.66	3.83	3.59
(β')	4.57	3.89	3.65	3.58	3.59
(″)	4.56	3.62	3.54	3.66	3.93
DMM					
α	5.09	3.80	3.85	3.90	4.02
β	4.78	3.84	3.63	3.81	3.67 [b]
(α')	4.57	3.90	3.64	3.82	3.63 [b]
(β')	4.53	3.88	3.67	3.82	3.63 [b]
(Δ)	5.01	3.82	4.30	5.64	–
GGG					
α	5.11	–	–	–	4.51
β	4.77	3.51	4.01	3.93	4.29
(′)	4.93	3.80	3.95	4.02	4.36
(″)	4.90	3.81	3.81	4.02	4.29
DGG					
α	5.12	–	–	–	4.52
β	4.78	3.50	4.00	3.91	4.30
(′)	4.90	3.76	4.00	4.13	4.36
(Δ)	5.08	3.81	4.23	5.75	–

[a] The prime (′) indicates the residue closest to the reducing end: (″) refers to the non-reducing end glycosyluronic unit; (Δ) refers to the unsaturated non-reducing end.
[b] Assignments may be reversed.

assignment was achieved by 2D proton–proton correlation and 1D TOCSY experiments (Table 1). In the case of β-mannose residue which displays a weak spin coupling constant, a weak magnetisation transfer was observed and it was necessary to increase the number of scans. Interestingly, the mannuronate and guluronate series exhibited a marked difference in their anomeric equilibrium. In both saturated and unsaturated oligomannuronates, the α/β ratio was about 2.2, whereas in oligoguluronates the β-form was largely predominant ($\alpha/\beta \simeq 0.2$). So, only the chemical shifts attributed to H-1(α) (5.11–5.12 ppm) and H-5(α) (4.51–4.52 ppm), which can be easily identified and useful for the determination of the structure of oligoalginates in this study, are given in the guluronate series. A double peak at 4.77–4.78 ppm, with a large coupling constant (6.3 Hz) is seen for H-1(β). In the unsaturated trimer of the guluronate series (ΔGG), the H-1 signal of the central residue resonates at 4.90 ppm and the attribution of the other protons is unambiguous. Concerning the GGG oligomer, the assignment of the signals at 4.90 and 4.93 ppm was not straightforward. Assuming the same chemical shift

for the H-5′ signal (4.36 ppm) in the saturated and unsaturated guluronate series, a correlation between the H-1 signal at 4.93 ppm and the H-5 signal at 4.36 ppm was observed in 1D TOCSY experiments. Proton attribution of central and non-reducing end units (Table 1) was deduced from the above criterion. It is also noteworthy that, as in the proton NMR spectra of unsaturated oligoglucuronates [29], the signals of the H-4 protons was found in the down-field region, at chemical shifts that are significantly

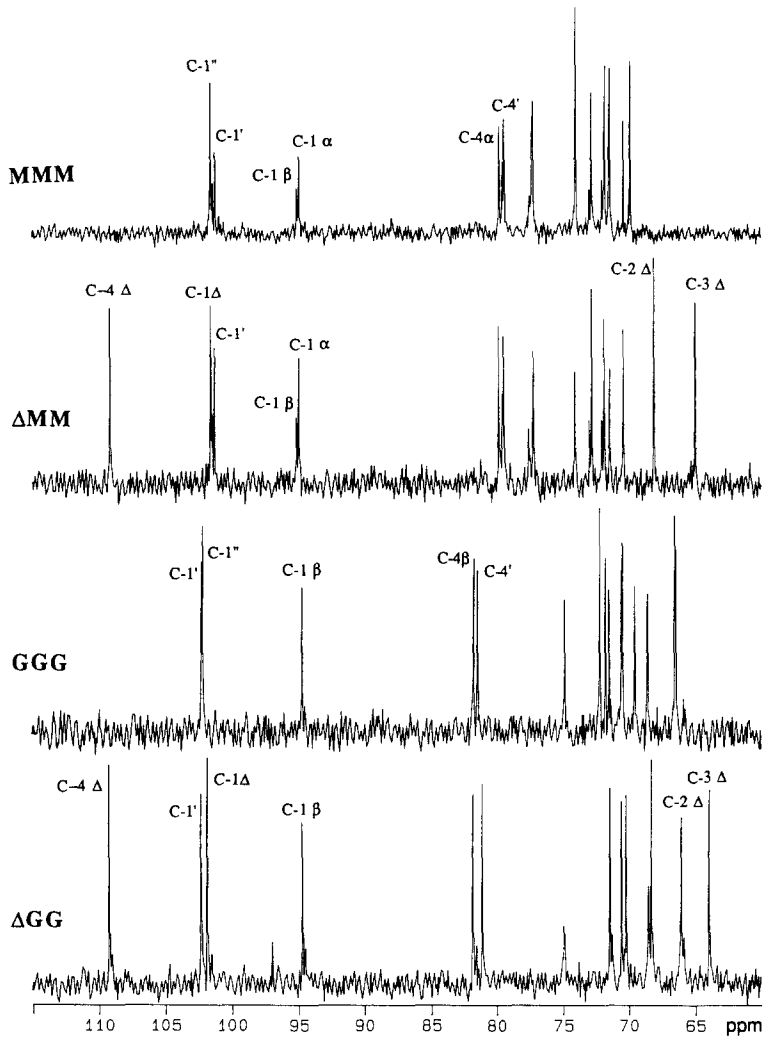

Fig. 2. ^{13}C NMR (75 MHz) spectra of solutions (20 mg mL^{-1}) in D_2O at pD 7 and 45 °C of sodium salts of various trimers obtained by acid or enzymatic hydrolysis of poly-mannuronate and -guluronate alginate blocks. The most relevant signals are pointed out as follows: the prime (′) indicates the residue closest to the reducing end; (″) refers to the non-reducing end glycosyluronic unit; (Δ) refers to the unsaturated non-reducing end.

Table 2
^{13}C NMR chemical shifts of oligoalginate trimers obtained by acid and enzymatic hydrolysis of poly-mannuronate and -guluronate blocks

	Chemical shifts (ppm) [a]				
	C-1	C-2	C-3	C-4	C-5
MMM					
α	94.68	71.23	70.21	79.65	73.92
β	94.92	71.79	72.76	79.26	77.09
(′)	101.03	71.59	72.63	79.34	76.96
(″)	101.36	69.70	73.82	77.17	71.23
DMM					
α	94.70	71.25	70.22	79.69	73.98
β	94.91	71.81	72.78	79.33	77.43
(′)	101.06	71.64	72.65	79.40	76.99
(Δ)	101.29	67.90	64.69	108.71	146.30
GGG					
β	94.52	70.39	71.30	81.42	74.67
(′)	101.94	66.38 [b]	70.39	81.15	68.43
(″)	101.85	66.44 [b]	71.63	71.92	69.42
DGG					
β	94.52	70.41	71.25	81.50	74.67
(′)	101.99	65.97	70.11	80.90	68.32
(Δ)	101.63	68.05	63.82	108.86	145.90

[a] The prime (′) indicates the residue closest to the reducing end; (″) refers to the non-reducing end glycosyluronic unit; (Δ) refers to the unsaturated non-reducing end.
[b] Assignments may be reversed. Chemical shifts of C-6 carbons were not considered in this study.

different between the mannuronate and oligoguluronate series, 5.64 and 5.75 ppm, respectively [12,24].

The 75-MHz ^{13}C NMR spectra of MMM, ΔMM, GGG, and ΔGG are given in Fig. 2. Chemical shifts were unambiguously assigned, using a HMQC pulse mode (Table 2). For the unsaturated oligoguluronate ΔGG, our assignments are in agreement with results of Natsume et al. [24]. For the unsaturated oligomannuronate ΔMM, however, we propose from ^{1}H–^{1}H COSY, 1D TOCSY and ^{1}H–^{13}C COSY experiments the sequence C-3α, C-2α, C-2′ up to low-field, whereas the assignments suggested by Natsume et al. were C-2α, C-2′ and C-3α [24]. The ^{13}C NMR spectra of saturated oligomannuronates and -guluronates with longer chain lengths (Tables 3 and 4) were resolved by comparison with the signals of tri-uronides, using the data reported by Grasdalen et al. [8].

Application to the determination of the substrate specificity of H. tuberculata *alginate lyase.*—Breakdown of alginates by alginate lyases results in the production of reducing ends and of unsaturated non-reducing ends. Based on Table 1 data, examination of the region between 4.7–4.9 ppm of a proton NMR spectrum of the reaction products would lead to the unequivocal structure determination of the reducing ends, indicating which uronic acid residue provides the anomeric hydroxyl group resulting from the endolysis of the glycosidic bond. In contrast, since β-elimination on β-D-man-

Table 3
^{13}C NMR chemical shifts of oligomannuronates obtained by acid hydrolysis of M blocks

	Chemical shifts (ppm) [a]				
	C-1	C-2	C-3	C-4	C-5
dp2					
α	94.76	71.31	70.22	79.71	73.98
β	94.91	71.82	72.81	79.46	77.17
(α′)	101.12	69.78	73.84	76.95	71.26
(β′)	101.33	69.75	73.84	76.95	71.23
dp3					
α	94.68	71.23	70.21	79.65	73.98
β	94.92	71.79	72.76	79.26	77.09
(′)	101.03	71.59	72.63	79.34	76.96
(″)	101.36	69.70	73.82	77.17	71.23
dp4					
α	94.68	71.19	70.21	79.67	73.96
β	94.93	71.79	72.77	79.27	77.11
(′)	101.04	71.58	72.62	79.28	77.08
(″)	101.27	71.47	72.62	79.24	77.11
(‴)	101.37	69.70	73.82	77.32	71.23
dp5					
α	94.65	71.20	70.24	79.74	74.06
β	94.95	71.78	72.79	79.47	77.13
(′)	101.06	71.43	73.63	79.36	77.01
(″)	101.30	71.43	73.63	79.24	76.91
(‴)	101.30	71.43	73.63	79.30	76.91
(⁗)	101.41	69.71	73.81	77.12	71.24
dp(n)					
	101.28	71.44	72.60	79.28	77.00

[a] The prime (′) indicates the residue closest to the reducing end; (″) refers to the β-D-mannosyluronic residue attached to the former unit and so on; dp(*n*) corresponds to M blocks before hydrolysis. Chemical shifts of C-6 carbons were not considered in this study.

nuronosyluronate or α-L-gulopyranosyluronate residues results in the same 4-deoxy-L-*erythro*-hex-4-enepyranosyluronate end group, one cannot identify the uronic acid residues initially involved at the other end of the linkage. Yet, as pointed out in Tables 5 and 6, several ^{1}H and ^{13}C resonances in the unsaturated ends are markedly dependent on the chemical nature of the nearest neighbour. One can therefore take advantage of the proton NMR spectroscopy characteristics of oligoalginates in the 5.5–5.8 ppm region in order to derive information on the nature of the polymeric sequences that were the preferential targets of β-elimination.

As an example of the interest of such an approach for investigating the substrate specificity of alginate lyases, degradation of polymannuronate, polyguluronate blocks and alginate by an abalone mannuronate lyase was monitored by ^{1}H NMR spectroscopy. Fig. 3 shows the ^{1}H NMR spectra of M and G blocks before and after addition of *H.*

Table 4
^{13}C NMR chemical shifts of oligoguluronates obtained by acid hydrolysis of G blocks

	Chemical shifts (ppm) [a]				
	C-1	C-2	C-3	C-4	C-5
dp2					
β	94.37	70.19	71.11	81.31	74.62
(′)	101.94	66.32	71.34	71.76	69.10
dp3					
β	94.52	70.39	71.30	81.42	74.67
(′)	101.94	66.38 [b]	70.39	81.15	68.43
(″)	101.85	66.44 [b]	71.63	71.92	69.42
dp4					
β	94.54	70.23	71.41	81.42	74.69
(′)	102.18	66.45	70.37	81.35	68.41
(″)	102.07	66.45	70.37	81.35	68.41
(‴)	102.07	66.45	71.63	71.92	69.30
dp6					
β	94.56	70.37	71.32	81.20	74.48
(′),(″),(‴),(⁗)	101.89	66.46	70.35	81.15	68.43
(⁗)	101.89	66.46	71.63	71.74	69.40
dp(n)					
	101.84	66.47	70.35	81.08	68.44

[a] The prime (′) indicates the residue closest to the reducing end; (″) refers to the α-L-gulosyluronic residue attached to the former unit and so on; dp(n) corresponds to the G blocks before hydrolysis.
[b] Assignments may be reversed. Chemical shifts of C-6 carbons have not been considered in this study.

Table 5
^{1}H NMR chemical shifts of the 4-deoxy-L-*erythro*-hex-4-ene residue in relation with the nature of its nearest neighbour in unsaturated alginate trimers

Linked to	Chemical shifts (ppm)			
	H-1	H-2	H-3	H-4
Mannuronate	5.01	3.82	4.30	5.64
Guluronate	5.08	3.81	4.23	5.75

Table 6
^{13}C NMR chemical of the 4-deoxy-L-*erythro*-hex-4-ene residue in relation with the nature of its nearest neighbour in unsaturated alginate trimers

Linked to	Chemical shifts (ppm)				
	C-1	C-2	C-3	C-4	C-5
Mannuronate	101.29	67.90	64.69	108.71	146.30
Guluronate	101.63	68.05	63.82	108.86	145.90

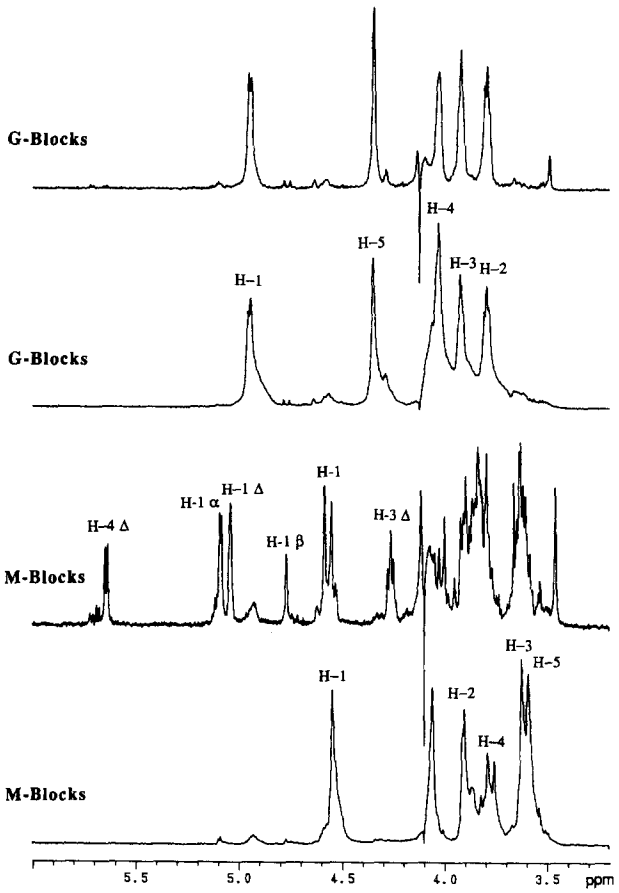

Fig. 3. ^{1}H NMR spectroscopy monitoring of the action of *H. tuberculata* alginate lyase on homopolymeric alginate blocks. Spectra were recorded at 300 MHz, 80 °C for poly-mannuronate (M) and -guluronate (G) blocks (10 mg mL^{-1}) before (lower spectrum) and after 15 min incubation (upper spectrum) at room temperature in the presence of 8 U mL^{-1} of lyase.

tuberculata partially purified lyase. The enzyme reaction was performed directly in D_2O and spectra were recorded after 15 min of incubation. Since the spectrum of G blocks remained unchanged while M blocks were degraded into a mixture of saturated and unsaturated oligouronides, the enzyme is clearly acting as a lyase specific for the poly-M homopolymeric regions, a result consistent with previously reported kinetic investiga-

Fig. 4. (a) ^{1}H NMR spectroscopy monitoring of the action of *H. tuberculata* alginate lyase on alginate. Spectra were recorded at 300 MHz, 80 °C for *Macrocystis pyrifera* alginate (10 mg mL^{-1}) and after 15 min incubation (upper spectrum) at room temperature in the presence of (from bottom to top) 0, 2, 8 and 12 U mL^{-1} of lyase. (b) Signal assignment of the most relevant protons in the ^{1}H NMR spectrum of the end products of *H. tuberculata* lyase (15 min incubation in the presence of 12 U mL^{-1} of lyase). Underligning refers to the considered unit.

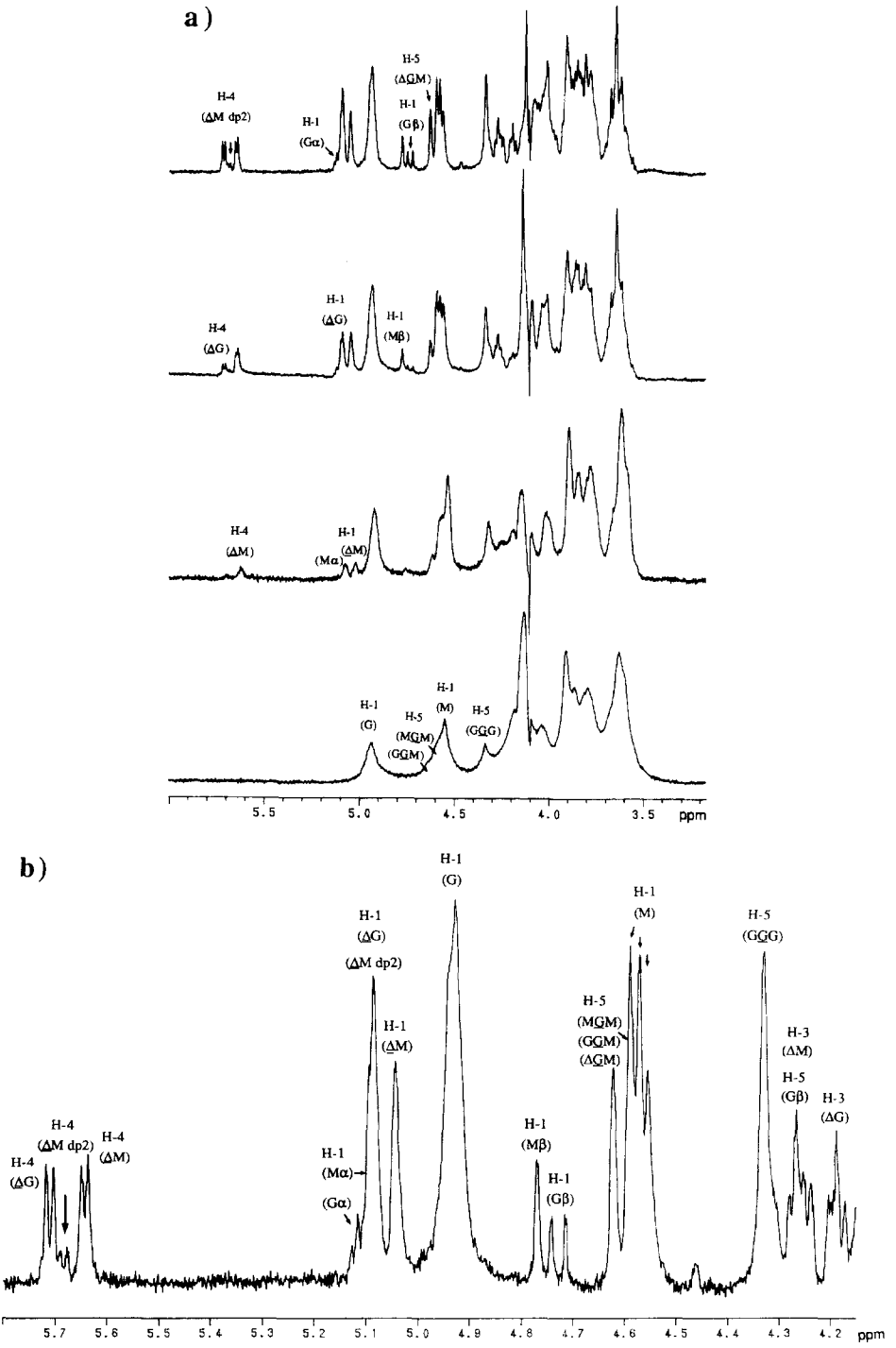
a)
H-4
(ΔM dp2)
H-1
(Gα)
H-5
(ΔGM)
H-1
(Gβ)
H-1
(ΔG)
H-4
(ΔG)
H-1
(Mβ)
H-4
(ΔM)
H-1
(Mα)
H-1
(ΔM)
H-1
(G)
H-5
(MGM)
(GGM)
H-1
(M)
H-5
(GGG)
5.5
5.0
4.5
4.0
3.5
ppm
b)
H-1
(G)
H-1
(ΔG)
(ΔM dp2)
H-1
(M)
H-5
(GGG)
H-5
(MGM)
(GGM)
(ΔGM)
H-3
(ΔM)
H-5
(Gβ)
H-3
(ΔG)
H-4
(ΔM dp2)
H-4
(ΔG)
H-4
(ΔM)
H-1
(Mα)
(Gα)
H-1
(Mβ)
H-1
(Gβ)
H-1
(ΔM)
5.7
5.6
5.5
5.4
5.3
5.2
5.1
5.0
4.9
4.8
4.7
4.6
4.5
4.4
4.3
4.2
ppm

Table 7
^{1}H NMR pertinent signals for the identification of alginate lyase products. Underlining refers to the considered unit and r refers to the reducing end position

Remarkable signals	H-1 and H-4($\underline{\Delta G}$)	H-1 and H-4($\underline{\Delta M}$)	H-1(G_β)	H-1(M_β)	H-5($\Delta\underline{G}M$)
Identified sequence	ΔG	ΔM	Gr	Mr	ΔGM
Possible origin [a]	G–MG	M–MM	G–M	M–M	G–MGM
	M–GG	M–GM		M–G	M–GGM
	M–MG	G–MM			M–MGM

[a] Hyphens indicate the site of β-elimination.

tions [13,14,19–21]. Neglecting the contribution of saturated non-reducing ends, integration of the appropriate signals of anomeric protons indicates an average polymerisation degree of 3 (DMM) for the end-produced unsaturated oligomannuronates. Similarly, Fig. 4a shows the ^{1}H NMR monitoring of the degradation of a commercial alginate sample from *Macrocystis pyrifera*, using various enzyme–substrate ratios. Fig. 4b points out the most relevant protons in the ^{1}H NMR spectrum of the end products. Attack of M blocks is first observed, with the appearance of new peaks corresponding to the anomeric protons of mannuronate reducing ends (Mr) and to the H-4 and H-1 protons of mannuronate-linked unsaturated ends (DM). At higher enzyme–substrate ratios, oligomers with a G unit at the reducing end (G_r) as well as a marked increase of the signal corresponding to DG oligouronides are also observed. Assuming, from its behavior in the presence of homopolyguluronate sequences (Fig. 3), that the lyase is not capable of breaking down GG linkages, the possible sequences that can account for the oligomeric entities generated upon enzymatic hydrolysis are listed in Table 7. These results are consistent with the attack of both GM and MM diads. Altogether, the alginate lyase of *H. tuberculata* therefore appears capable of performing β-elimination on mannuronate residues, whatever their immediate neighbours, as proposed previously from kinetic data by Østgaard et al. [30,31]. The improvement of the resolution of the spectrum of G blocks upon incubation in the presence of M-lyase (Fig. 3) is therefore likely to account for by the breakdown of the $\sim$ 5% mannuronate units initially present in this substrate.

In conclusion, the complete ^{1}H NMR spectroscopy characterisation of various saturated or unsaturated oligouronates now makes possible the direct, unequivocal identification of the reducing end residues as well as of the residues closest to the unsaturated, non-reducing ends in the end products of alginate lyases. The feasability of this approach for a quick analysis of the substrate specificity of alginate lyases and, eventually, for the determination of alginate chemical composition [20,21] is illustrated by the monitoring of alginate degradation by *H. tuberculata* alginate lyase. As previously reported from chromatographic and kinetic analyses [25,30,31], ^{1}H NMR spectroscopy analysis of the degradation products shows that the enzyme presents a high specificity for MM homopolymeric sequences and is not capable of breaking down polyguluronate blocks. It also confirms that the abalone lyase is capable of β-elimination on mannuronate residues independently of their immediate sequence environment. In this respect the abalone lyase cannot be referred to as a polymannuronate lyase since

its ability to recognise mannuronate residues in alginate is not dependent upon the presence of homopolymeric mannuronate sequences.

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