Carbohydrate Research, 191 (1989) 167–173
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Composition and sequence of uronate residues in alginates from three species of brown seaweeds

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(Received August 23rd, 1988; accepted for publication, February 24th, 1989)

Sodium alginate is a linear glycuronan which consists of (1→4)-linked residues of β-D-mannuronate (M) and α-L-guluronate (G) residues arranged in a blockwise fashion. Homopolymeric (MM and GG) blocks are separated by heteropolymeric (MG and GM blocks)[1–3].

The n.m.r. signals due to H-1 and H-5 in the G residues and H-1 in the M residues can be distinguished[4] in homopolymeric blocks as can that of H-5 of GM and GG[5]. Thus, the relative proportions of the sequences MM, GG, MG, and GM can be obtained. The relative proportions of M- (MMM, GMG, MMG, and GMM) and G-centred triads (GGG, MGM, GGM, and MGG) were obtained by ^{13}C-n.m.r. spectroscopy[6,7], and ^{1}H-n.m.r. spectroscopy at 400 MHz was used to obtain information about G-centred triads[8].

Physical properties, such as gelling characteristics of alginates, depend upon the M/G ratio and the relative proportions of the MM, GG, and MG/GM blocks[1]. For example, alginates with a low M/G ratio or a relatively higher content of G residues form strong brittle gels which tend to synerese in the presence of excess of Ca^{2+}, whereas alginates with a high M/G ratio or a higher content of M residues are tolerant[4] to high levels of Ca^{2+}.

We now describe the application of ^{1}H-n.m.r. and ^{13}C-n.m.r. spectroscopy to alginates isolated from the brown seaweeds *Turbinaria conoides*, *Cystoseira trinodis*, and an unidentified species of *Sargassum*.

The yields of calcium alginate (Table I) show that the three species of brown seaweeds are good sources. The direct extraction procedure gave better yields and was less tedious. The ^{1}H- and ^{13}C-n.m.r. spectra of the partially degraded alginate samples, interpreted using literature data, indicated that each was rich in G residues.

The ^{1}H-n.m.r. spectrum (Fig. 1) of the partially degraded alginate sample obtained by sequential extraction of *T. conoides* is representative of the spectra obtained for the other alginates, except for the differences in the intensities of the peaks. The signals A and C are due to H-1 of G residues and H-5 of GM residues,

0008-6215/89/$ 03.50

TABLE I

YIELDS (%) OF CALCIUM ALGINATE ISOLATED[a]

Seaweed	*Yield (%)*	*Yield (%)*
	Sequential extraction	*Direct extraction*
T. conoides	24.2	79.5
C. trinodis	29.4	87.1
Sargassum sp. (linear)	38.8	Not determined

[a]G/100 g of dry matter.

respectively, whereas the signal B at δ 4.7 is due to both H-1 of M residues and H-5 of GM residues[4,5].

The M/G ratios as well as the doublet frequencies (Table II) were calculated[5]. The low-field region of the ^{1}H-n.m.r. spectrum in Fig. 1 contains a dominant A peak that is in keeping with the low M/G ratio.

The ^{1}H-n.m.r. analysis of the three alginates, isolated by the sequential extraction procedure, shows that they have M/G ratios of <1, indicating the presence of relatively large proportions of G residues. The results also indicate

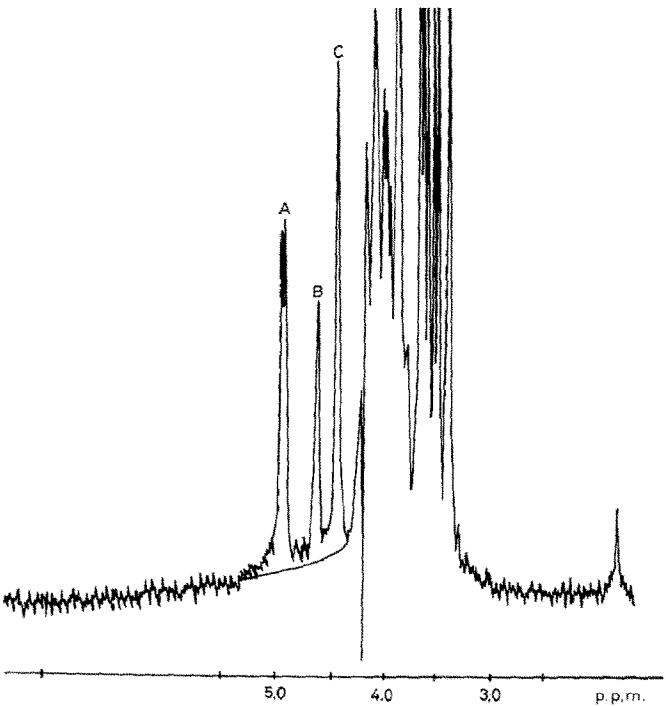

Fig. 1. ^{1}H-N.m.r. spectrum (100 MHz) of the depolymerised sodium alginate sample from *T. conoides*.

TABLE II

COMPOSITION, DOUBLET FREQUENCIES, AND M/G RATIOS OF SODIUM ALGINATE SAMPLES BY ^{1}H-N.M.R. ANALYSIS

Seaweed	*Method of extraction*	*Composition*		*Doublet frequencies*				*M/G ratio*
		F_G	F_M	F_{GG}	F_{GM}	F_{MG}	F_{MM}	
T. conoides	S[a]	0.76	0.24	0.65	0.11	0.11	0.13	0.32
	D	0.66	0.34	0.57	0.09	0.09	0.25	0.52
C. trinodis	S	0.63	0.37	0.58	0.05	0.05	0.32	0.59
	D	0.58	0.42	0.55	0.03	0.03	0.39	0.72
Sargassum sp. (linear)	S	0.67	0.33	0.59	0.08	0.08	0.25	0.49

[a]S, sequential; D, direct extraction.

higher contents of GG than M sequences. The GM and MG sequences occur to a small extent. These results were confirmed by ^{13}C-n.m.r. analysis of some of the samples (Table III).

The ^{13}C-n.m.r. spectrum (Fig. 2) for the partially depolymerized alginate sample of *T. conoides* is representative of the spectra of the other alginates examined except for differences in peak intensities.

Assignments of the ^{13}C-n.m.r. resonances of the alginates were made by comparison with reported data[7]. The diad sequences MM, GG, MG, and GM were obtained from the C-1 signals. The C-5 signal of M residues apparently was sensitive to both nearest neighbour residues and it was possible to analyse the M-centred triads using the C-1, C-4, and C-5 resonances. The M/G ratios and the M-centred triad frequencies were then calculated[7] (Table III). However, it was not possible to obtain data for the G-centred triad sequences, as the C-1 and C-6 resonances were incompletely resolved at 25 MHz.

The results indicate that the alginates studied are composed of large G blocks, shorter M blocks, and a small proportion of GM blocks. Alginates that contain large proportions of G residues are not common in Nature. Hydrolysis of the G–M bond occurs more easily than for the M–G bond during exhaustive extraction procedures. Hence, a direct extraction procedure was also used to isolate the alginates, and ^{1}H-n.m.r. spectroscopy (Table II) indicated them to be rich in G residues. The increase in the M/G ratios suggested that some degradation of M residues occurred during the sequential extraction procedure. Therefore, the three alginates examined were rich in G residues and GG-sequences.

Alginates with M/G ratios of <1, which are also rich in GG sequences, form gels in the presence of Ca^{2+} and are of use in the food, textile, and cosmetic industries as emulsion stabilizers and thickening agents.

TABLE III

COMPOSITION AND DIAD AND TRIAD FREQUENCIES IN PARTIALLY DEGRADED ALGINATES[a] BY ^{13}C-N.M.R. SPECTROSCOPY

Seaweed	F_G	F_M	F_{GG}	F_{GM}	F_{MG}	F_{MM}	F_{MMM}	F_{MMG}	F_{GMM}	F_{GMG}	*M/G ratio*
T. concoides	0.75	0.25	0.69	0.06	0.06	0.19	0.15	0.04	0.04	0.02	0.33
C. trinodis	0.59	0.41	0.40	0.19	0.19	0.22	0.38	0.06	0.06	0.13	0.69
Sargassum	0.66	0.34	0.59	0.07	0.07	0.27	0.02	0.05	0.05	0.02	0.52

[a]Obtained by the sequential extraction procedure.

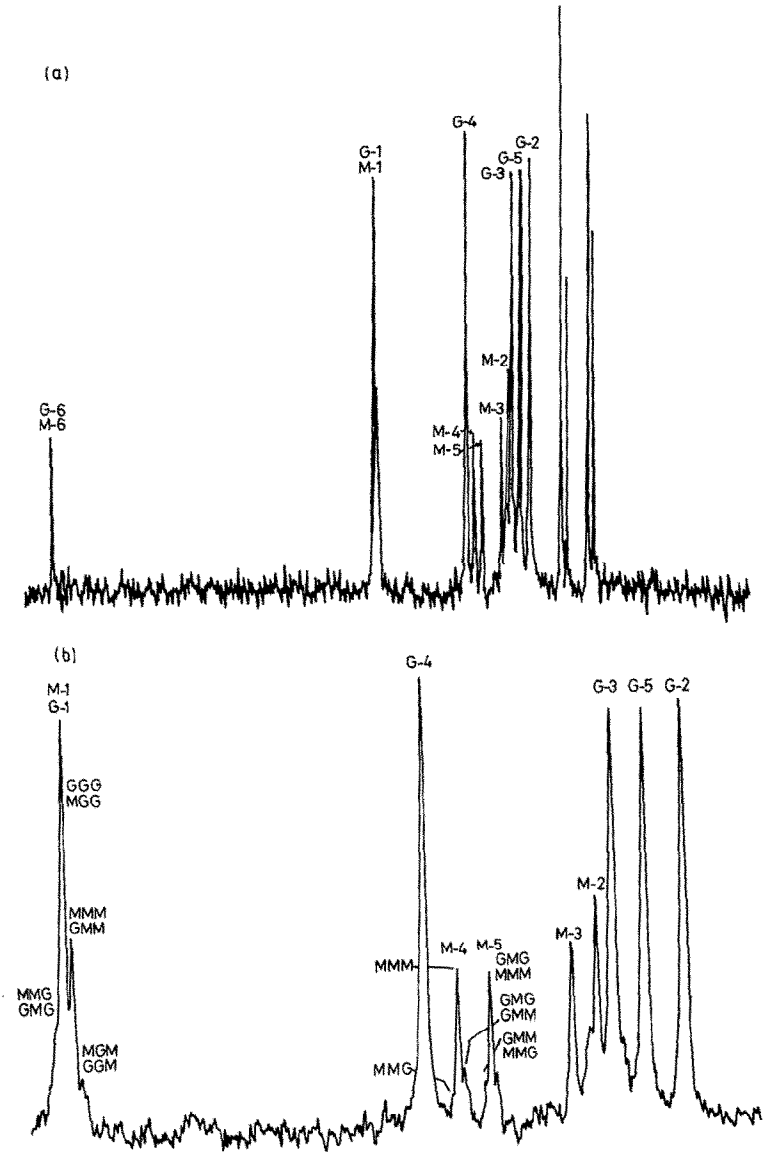

Fig. 2. (a) ^{13}C-N.m.r. spectrum (25.2 MHz) of the depolymerised sodium alginate from *T. conoides;* (b) expansion of the signals due to C-1,4,5,3,2 of the G and M residues in the depolymerised sodium alginate from *T. conoides.*

EXPERIMENTAL

The brown seaweeds *T. conoides*, *C. trinodis*, and an unidentified species of *Sargassum*, referred to as *Sargassum* sp. (linear) with respect to the shape of its fronds, were collected from the coastal regions of Sri Lanka. Alginates were isolated as follows.

Sequential extraction. — Milled seaweed (200 g) was extracted successively with aqueous 80% ethanol (2 × 1 L), first at room temperature (2 × 6 h), then at 70° (2 × 4 h). Each seaweed residue was treated overnight with aqueous 40% formaldehyde and then extracted in sequence with aqueous 2% $CaCl_2$ (1 L) at room temperature (8 h), aqueous 2% $CaCl_2$ (1 L) at 70° (8 h), dilute HCl (pH 2, 1 L) at room temperature (8 h), dilute HCl (1 L) at 70° (8 h), and aqueous 3% Na_2CO_3 (1 L) at 50° (6 h). The last extract was poured with stirring into 4 vol. of ethanol, the precipitate was collected, and a solution in water was dialysed and freeze-dried. A solution of the resulting white powder in a small volume of water was stirred with aqueous 2% $CaCl_2$ until precipitation was complete, to give calcium alginate.

Direct extraction. — Milled seaweed (10 g) was treated with aqueous 1.8% formaldehyde for 30 min, the supernatant solution was discarded, the residue was suspended in water (300 mL), and Na_2CO_3 (3.0 g) and aqueous 0.1% NaOH (3.0 mL) were added. The mixture was stirred for 2 h, filtered, neutralized with dilute HCl, and then diluted to 1.6 L with water. Aqueous $CaCl_2$ solution (100 mL) was then added with stirring to bring the concentration of $CaCl_2$ to 2%. The gelatinous calcium alginate precipitate was separated by centrifugation, washed with dilute aqueous $CaCl_2$ solution, suspended in water, and freeze-dried. The seaweed residue from above was re-extracted with aqueous 3% Na_2CO_3 (3 × 300 mL) at 70° (3 × 3 h). The combined extracts were treated as described above, to give a white precipitate of calcium alginate.

Conversion of calcium alginate into sodium alginate. — The calcium alginate precipitate was suspended in 0.5M HCl and stirred occasionally, and, after 3 h, the alginic acid was collected, washed with 0.5M HCl until free from Ca^{2+} ions, suspended in water, and titrated to pH 7 with 0.1M NaOH. The resulting solution was dialysed for 48 h against distilled water to give sodium alginate.

N.m.r. spectroscopy. — Solutions of sodium alginate (100 mg) in water were dialyzed against distilled water containing 1% of EDTA for 24 h, then freeze-dried. Each purified sodium alginate (50 mg) was dissolved in water (50 mL), the pH of the solution was adjusted to 3 with 0.1M HCl, the mixture was heated at 100° for 30 min, the pH was adjusted to 7, and the solution was freeze-dried to give the partially degraded sodium alginate.

A solution of each of these products (10 mg) in D_2O (0.5 mL) containing EDTA (3 mg) was used for 100-MHz ^{1}H-n.m.r. spectroscopy with a JEOL FX-100 or JEOL FX-90Q spectrometer. The chemical shifts are expressed in p.p.m. downfield from that of internal sodium 4,4-dimethyl-4-silapentanesulfonate. The peak areas were measured by planimetry.

To a solution of each partially degraded sodium alginate (100 mg) in D_2O (1 mL) at pH 7 was added sodium triethylenetetra-aminehexa-acetate (25 mg/mL)[7]. The ^{13}C-n.m.r. spectra were then recorded with a JEOL FX-100 (25 MHz) or JEOL FX-90Q (22.5 MHz) spectrometer at 90°. The chemical shifts are expressed in p.p.m. relative to that of internal sodium 3-trimethylsilyltetradeuteriopropionate. The peak areas of all the partly overlapping peaks were measured by planimetry.

The M/G ratios, together with doublet and triplet frequencies, were calculated for whole alginate samples by ^1H- and ^{13}C-n.m.r. spectroscopy by using peak areas for quantification.

In order to obtain the most accurate values for the diads and M-triads, the relationship between M-triads and the intensities was written[7] and numerical values for the triads were substituted from the spectra.

ACKNOWLEDGMENTS

We thank Dr. H. Grasdalen (Institute of Biotechnology, Trondheim, Norway) for his help and, together with Dr. M. I. M. Wazeer (University of Petroleum and Minerals, Dharan, Saudi Arabia), for the ^1H- and ^{13}C-n.m.r. data, the Swedish University of Agricultural Sciences (Uppsala) for facilities, the International Foundation for Science (Stockholm) for a research grant, and the International Seminar in Chemistry (Uppsala University) for a research fellowship (to S.S.M. de S.). This work was suported by the Natural Resources, Energy, and Science Authority of Sri Lanka.

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