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Note

High-field, ^{1}H-n.m.r. spectroscopy of alginate: sequential structure and linkage conformations

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As a continuation of n.m.r. studies of the composition and the sequence of uronate residues in intact alginates[1–5], we now report on 400-MHz, ^{1}H-n.m.r. spectra of alginates and alginate fractions. These spectra provide information about guluronate(G)-centred triads previously accessible only from high-field, ^{13}C-n.m.r. spectroscopy[4], and support some predictions about linkage conformations based upon hard-sphere calculations[6,7].

The preparations of the alginates, alginate fractions[8,9], and n.m.r. samples[4] have been reported. Fig. 1 shows the anomeric region in the 400-MHz, ^{1}H-n.m.r. spectra of two different fractions of alginate. According to our previous assignments[2], the two main peaks at 5.05 and 4.46 p.p.m. in Fig. 1A for an alginate fraction enriched in homopolymeric G-blocks are due to H-1 and H-5 of the G residues in these blocks, respectively. The minor peaks centred around 4.7 p.p.m. arise from H-5 of G residues adjacent to mannuronic acid (M) residues and from H-1 of M residues, which can be inferred from Figs. 1B and 2C, respectively. In the spectrum in Fig. 1B, which is for a fraction enriched in alternating sequences (-MGMGMG-), the latter peaks are quite dominant. Partial protonation of the carboxyl groups shifts the signals for H-5 downfield, as demonstrated in Fig. 1C recorded at pD 4. Evidently, H-5 of G gives four peaks. Since the ^{13}C-n.m.r. data[1] confirmed that the uronic acid residues exist in their normal chair conformations, regardless of their sequence, this can only be explained by a sequential splitting, thus providing the key for directly measuring the four G-triad frequencies.

In addition to primary structure and the chair conformations of individual residues, an important contribution to proton shifts in polysaccharide systems may arise from nuclear shielding due to the proximity of protons of electronegative groups. This "through-space" effect is sensitive to configurational structure about glycosidic bonds. Whittington reported[6,7] hard-sphere conformational-energy calculations on alginic acid and presented values for the angles (ϕ and ψ) determining the conformations of the four possible dimers. A comparison of the conformation of the glycosidic bond in GM (**1**) with that in the GG dimer (**2**) showed that,

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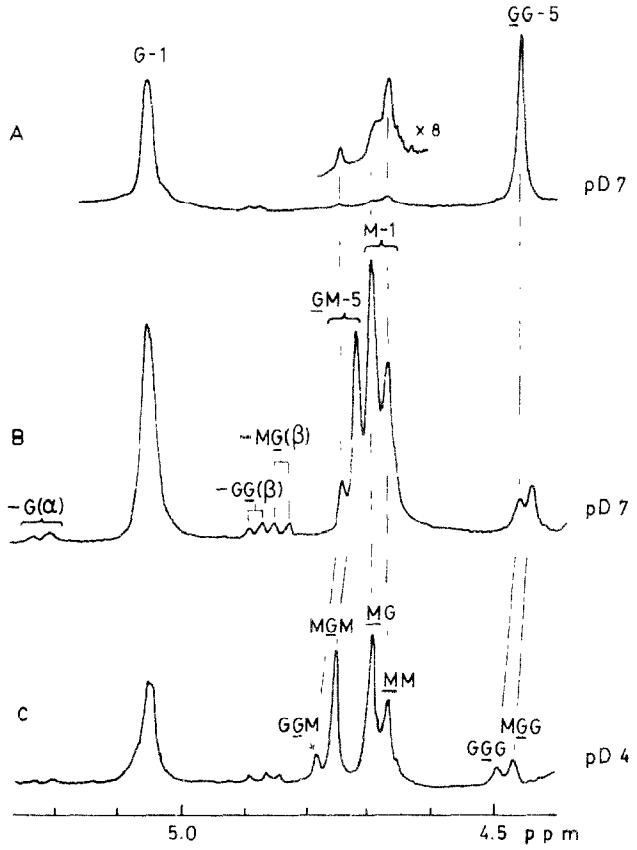
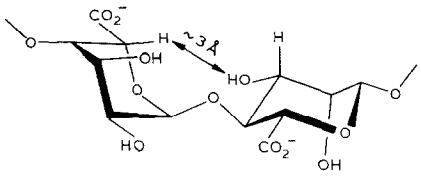


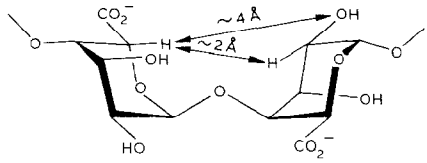
Fig 1. The anomeric region in the 400-MHz, ^{1}H-n m.r spectra of two different fractions of sodium alginate obtained by chemical fractionation. A, "G-fraction" enriched in L-guluronate (~90%), B, "MG-fraction" enriched in alternating sequence (M/G molar ratio ~1.2), in D_2O (5 mg/0.5 mL) at pD 7 and 90° (obtained with a Bruker WM-400 n m.r spectrometer), C, "MG-fraction" recorded at pD 4. The interpretation of the different peaks is indicated by underlining the units involved. M, mannuronate; G, guluronate.

whereas H-5 of G is in close proximity (~3 Å) to O-2 of M in the former, this proton comes closest (~2 Å) to H-2, and ~4 Å from O-2, of G in the latter. Hence, the H-5 of G resonating at ~4.7 p.p.m. most probably arises from the GM dimers, in which the proximity of the electronegative HO-2 group to the adjacent M residue affects the nuclear deshielding through space much more effectively than in the GG dimers. The small splittings (~0.02 p.p.m.) in the G(H-5) line-pattern must be induced through bonds by changing the residue linked to G-4, *i.e.*, the preceding unit in the chain.

The assignments in Table I can be made with certainty by considering the chemical composition of the alginate fractions. In Fig. 1A for the "G-fraction", the dominant H-5 peak of G must arise from the triad GGG, whereas, in the spectrum of the "MG-fraction", the main H-5 line of G originates from the triad MGM. The



1(GM)



2(GG)

positions of the asymmetric G-triads, MGG and GGM, are now self-evident in Fig. 1C. Irrespective of the residue linked to C-1, a preceding G-residue leads to a slightly more deshielded G(H-5) resonance. The M diads could also be directly measured in the 400-MHz, ^{1}H-n.m.r. spectrum. The resonance for H-1 of M in the diad MG is downfield (~0.03 p.p.m.) of that for H-1 of the diad MM, as shown in

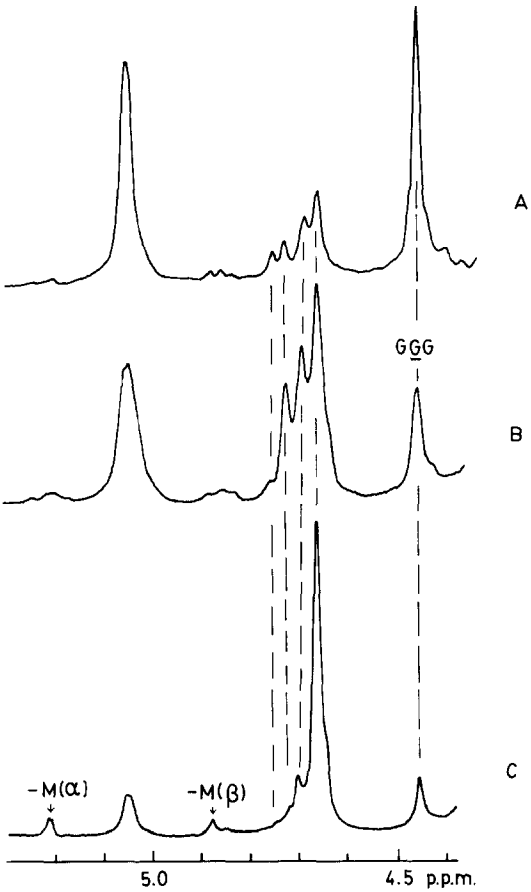


Fig. 2. The anomeric region in the 400-MHz, ^{1}H-n.m.r. spectra of the algal sodium alginates of A, *Laminaria hyperborea* stipe 4/5; B, *Macrocystis pyrifera;* and C, *Ascophyllum nodosum*, high-M alginate, recorded at 90°, pD 6.

TABLE I

CHEMICAL SHIFTS[a] OF PROTON RESONANCES FOR ALGINATE IN D_2O AT 90° (pD 6)

Residue	Sequence	Proton			
		H-1 (reducing end)		H-1 (internal)	H-5 (internal)
		(α)	(β)		
M	MM	5.21	4.89	4.67	
	MG			4.70	
G	GG		4.88	5.05	
	GM		4.84		
	GGG			5.05	4.46
	MGG			5.05	4.44
	GGM			5.05	4.75
	MGM			5.05	4.73

[a] In p.p.m. downfield from internal sodium 3-(trimethylsilyl)propionate d_4 for the resonance of the left-hand residue in the listed diads and of the intermediate residue in the listed triads.

Fig. 1C. The interpretation becomes evident upon comparison with the spectrum (Fig. 2C) of a high-M alginate sample.

Confidence in this interpretation is enhanced by using the proton-signal integrals, which reflect the quantities of the respective trequencies[2]. The intensities of the respective lines fulfil the following relationships, valid for long chains.

$$F_G = F_{GGG} + F_{MGG} + F_{GGM} = F_{MGM}$$

$$F_{MG} = F_{GM} = F_{GGM} + F_{MGM}$$

$$F_{MGG} = F_{GGM}$$

Because of the necessity for slight degradation of the polymers in order to obtain well-resolved n.m.r. spectra, some weak signals from the anomeric protons at the reducing end also appear, as shown in Figs. 1 and 2. Their resonance positions (Table I) show deshielding power through bonds, as for the internal units. For a reducing, terminal G-residue in the β-anomeric form, an adjacent G-unit attached to C-4 causes a downfield shift ($\Delta = 0.04$ p.p.m.) of the signal for H-1 relative to that for an MG reducing end-unit.

^{1}H-N.m.r. spectra of whole alginate samples at neutral pD are shown in Fig. 2. The positions of the lines can be recognised as corresponding to those in the spectra of alginate fractions. Since separate signals appeared for M diads and G triads, numerical values for their relative occurrence were measured directly. The results obtained for the alginate fractions, and for some alginates isolated from whole plants, are shown in Table II. The corresponding values derived from ^{13}C-

TABLE II

DISTRIBUTION OF DIAD AND TRIAD FREQUENCIES IN ALGINATE FRACTIONS AND WHOLE ALGINATES

Sample	*Method*[a]	F_{MM}	F_{MG} F_{GM}	F_{GG}	F_{GGG}	F_{GGM} F_{MGG}	F_{MGM}
"MG-fraction"	A	0.24	0.33	0.09	0.04	0.05	0.28
	B	0.23	0.33	0.11	0.05	0.06	0.27
"G-fraction"	A	0.08	0.02	0.88	0.86	0.02	~0
	B	0.05	0.05	0.85			
Laminaria digitata	A	0.43	0.15	0.27	0.22	0.05	0.10
	B	0.46	0.14	0.26	0.22	0.04	0.10
L. hyperborea	A	0.20	0.10	0.60	0.56	0.04	0.06
stipe 4/5	B	0.20	0.14	0.52			
Ascophyllum nodosum	A	0.32	0.25	0.19	0.13	0.06	0.17
old tissue	B	0.34	0.23	0.20	0.12	0.08	0.15
Macrocystis pyrifera	A	0.43	0.18	0.21	0.20	~0.01	0.20
	B	0.38	0.21	0.20	0.19	~0.01	0.20
Ascophyllum nodosum	A	0.42	0.15	0.28	0.24	0.04	0.11
cultured specimen	B	0.34	0.20	0.27	0.20	0.07	0.13

[a]A, ^{1}H-n.m.r. data (present work); B, ^{13}C-n.m.r. data.

n.m.r. data are also shown for comparison. In all cases, excellent agreement is found between the two sets of data.

The samples showed diad and triad frequencies in highly variable proportions. It should be noted that the MGM triad was absent from the structure of the G-enriched alginate fraction. This finding is reasonable from simple statistical considerations, since the content of M is only ~10%. As concluded previously[2,3], the character of the block structure is evident for whole alginates. In the alginate from *Macrocystis pyrifera,* an unusual sequential distribution of the G residues was found. They were about equally distributed among GGG and MGM triads, whereas only a very small fraction appeared in the asymmetric triads. The significance of this finding is that half of the G residues are located as single units flanked by M residues, whereas the rest occur as fairly long G-blocks. This type of averaged sequence may possibly be brought about by heterogeneity in the alginate molecules isolated from whole plants and/or as a result of the mechanism for the biosynthesis of the alginate molecules.

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