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^{13}C NMR Spectra at 67.9 MHz of Aqueous Solutions of Agarose and Partly 6-*O*-Methylated Agarose at 95 °C

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Based on chemical shifts and carbon/hydrogen coupling constants from model molecules, a detailed assignment of the observed chemical shifts is presented.

Polysaccharide gels have been the topic of numerous investigations.[1-4] However, although an intensive research aimed at the understanding of the gel formation and gel properties in molecular terms has been carried out in recent years, a detailed understanding is still not fully achieved.

The technological development in spectrometers has made ^{13}C NMR spectroscopy a powerful tool in the investigation of macromolecules [5,6] including polysaccharides. The reported results initiated our work with agarose, with the goal to contribute to the understanding of gel formation and gel properties.

Agarose is one of the simplest polysaccharides which may form gels. This fact, together with the facts that agarose forms very strong gels and that the gels show very pronounced gel characteristics such as hysteresis and syneresis[1] make agarose a key molecule to the study of many gel properties. Until now only a few papers dealing with ^{13}C NMR spectra of agarose-like polysaccharides have appeared in the literature,[7-10] but so far no detailed ^{13}C NMR investigation of agarose has been reported.

Below we present a 67.9 MHz ^{13}C NMR study of agarose/water solutions at elevated temperature. In subsequent papers we will report on the influence of temperature and gelation on the ^{13}C NMR spectra of agarose, as well as the results of ^{13}C relaxation measurements.

^{13}C NMR SPECTRA OF AGAROSE

The basic unit of agarose has the simple structure of a 3-linked β-D-galactopyranosyl residue (G in Fig. 1) and 4-linked 3,6-anhydro-α-L-galactopyranosyl residue (A in Fig. 1). The number of repeating units is several hundred. Each residue contains six chemically different carbon atoms which in the following will be named G1–G6 and A1–A6 for the galactopyranosyl and the anhydro galactopyranosyl, respectively. For commercially available agarose the major deviation from the structure given in Fig. 1 is G-residues which are 6-*O*-methylated. For the examinations described below two types of agarose from LITEX have been used. The two types are characterized by a very low and a relatively high (*ca.* 25 %) number of 6-*O*-methylated G-residues.

At temperatures above 70 °C it is relatively easy to obtain ^{13}C NMR spectra of aqueous solutions of agarose. Figs. 2 and 3 show the noise decoupled ^{13}C NMR spectra of the low and high methylated agarose recorded at 95 °C using 5 % solutions w/v.

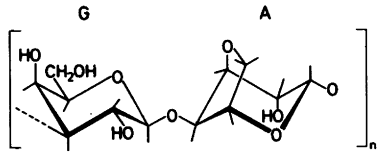

Fig. 1. The repeating unit of agarose. For clarity the hydrogen atoms in the rings are omitted.

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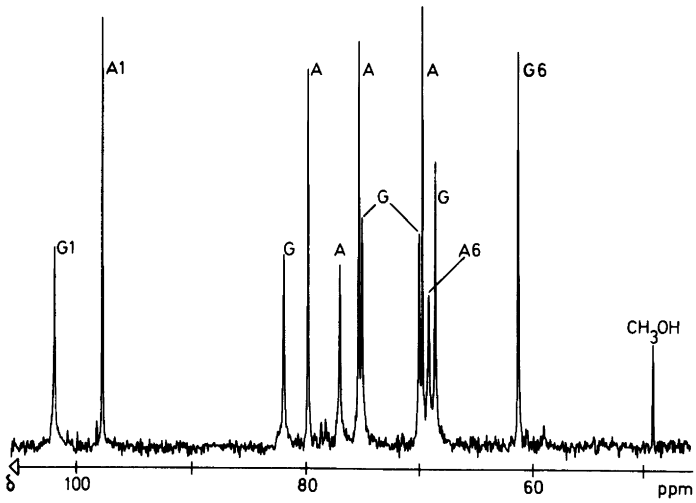

Fig. 2. ^{13}C NMR spectrum of agarose in water (5 %) at 95 °C.

The spectrum in Fig. 2 is dominated by 12 different signals as expected if the agarose consists entirely of the basic unit shown in Fig. 1.

Off resonance and gated decoupled spectra were also recorded. In Fig. 4 a gated decoupled spectrum of the low methylated sample is shown. From these spectra the number of hydrogens directly attached to each carbon atom and the value of the carbon-hydrogen coupling constants are obtainable. In Table 1 the chemical shifts for the individual resonance signals are listed together with the determined number of attached hydrogens (N) and the measured coupling constants, $^{1}J_{CH}$.

ASSIGNMENT OF CHEMICAL SHIFTS

As a support in the assignment, data from model compounds have been used. The most relevant ^{13}C NMR data obtainable were for methyl 3,6-anhydro-α-D-galactopyranoside [11] and for methyl 3-*O*-methyl-β-D-galactopyranoside.[12] The data for these compounds are collected in Table 2. In order to correct for the influence of the glycosidic bond in agarose,[12] 7 ppm was added to the chemical shift value for the 4-carbon atom in the model compound. For the remaining carbons minor deviations are to be expected.[12]

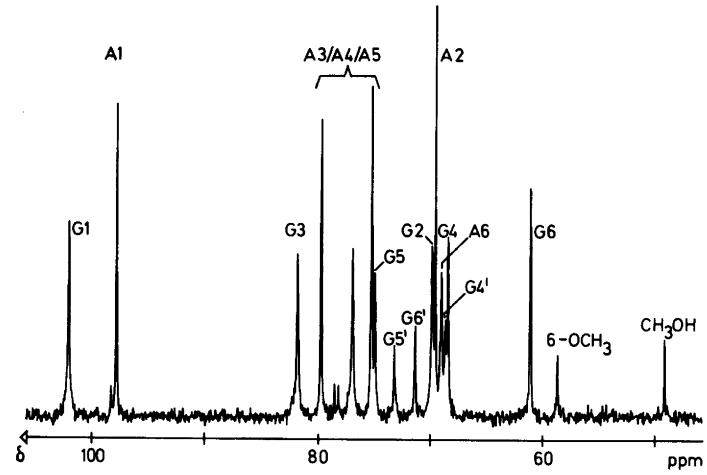

Fig. 3. ^{13}C NMR spectrum of partly 6-*O*-methylated agarose in water (5 %) at 95 °C.

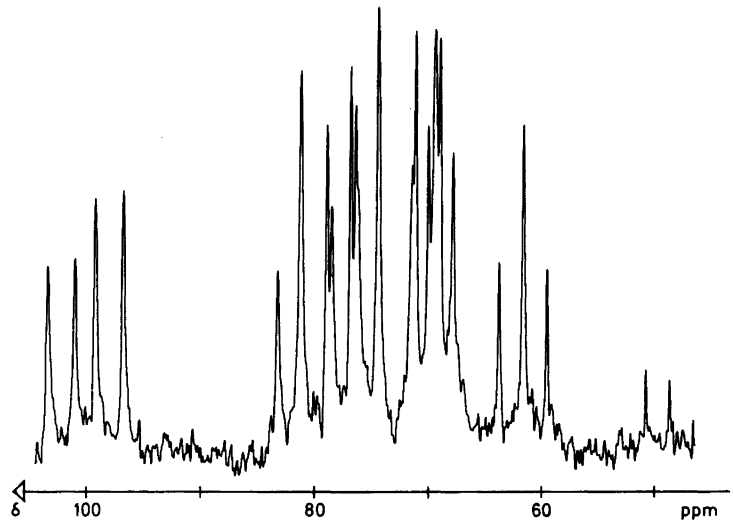

Fig. 4. Gated decoupled ^{13}C NMR spectrum of agarose in water. The sample used is the same as in Fig. 2.

From the data in Table 2 it appears that the value for the carbon hydrogen coupling constants with the exception of the C1 carbon atoms fall into two distinct groups for the two model substances. Thus the $^{1}J_{CH}$ coupling constants for the G2–G6 carbons can be expected in the range 140–150 Hz,[13,14] whereas the corresponding values for the A-residue can be expected in the region 150–160 Hz.[11] Also for the C1 carbons the value of the coupling constant for the A-residue can be expected to be larger than for the G-residue.

From the chemical shift and the coupling constants A1 and G1 can be assigned at 97.9 and 102.2 ppm, respectively. This is in close agreement with the data obtained by Yaphe *et al.* for agarose[8] and κ-carragenan.[9] The A6, G6, G6′ and the 6-OCH_3 carbons are also easily assigned based on data from the model compounds combined with the measured

Table 1. ^{13}C NMR data for agarose (Sample 1) and partly 6-*O*-methylated agarose (Sample 2).

Chemical shift/ppm		*N*	$^{1}J_{CH}$/Hz	Assignment (see text)	
Sample 1	Sample 2			I	II
102.2	102.2	1	162	G1	G1
97.9	97.9	1	168	A1	A1
81.9	81.9	1	~140	G	G3
79.9	79.9	1	158	A	
77.1	77.1	1	160	A	A3/A4/A5
75.4	75.4	1	162	A	
75.1	75.1	1	~140	G	G5
	73.3	1			G5′
	71.5	2		G6′	G6′
70.0	70.0	1	143	G	G2
69.7	69.7	1	152	A	A2
69.1	69.1	2	?	A6	A6
	68.8	1		G	G4′
68.6	68.6	1	149	G	G4
61.2	61.2	2	143	G6	G6
	58.8	3		6-OCH_3	6-OCH_3

Table 2. ^{13}C NMR data for agarose model molecules.

Atom	Methyl 3,6-anhydro-α-D-galactopyranoside [11]		Methyl 3-*O*-methyl-β-D-galactopyranoside	
	Chemical shift/ppm	$^1J_{CH}$/Hz	Chemical shift/ppm [12]	$^1J_{CH}$/Hz [13,14]
C1	98.6	165	103.9	160
C2	69.8	150	69.8	
C3	81.5	160	82.0	
C4	77.5[a]	157	64.2	143–149
C5	77.7	163	75.1	
C6	69.5	151	61.2	
OCH_3	58.0	144	56.2	
			57.3	

[a] Seven ppm added to the original value (see text).

value *N* of attached hydrogens given in Table 1. The carbon atoms in the 6-*O*-methylated G-residue is here and in the following named G1′, G2′, *etc.*

The remaining resonance lines can from the value of the carbon/hydrogen coupling constants be assigned to either the A or the G residue as shown in column I under assignment in Table 1.

We believe the assignment given so far is without ambiguity. The assignment is summarized in Table 1 in column I, and for the low methylated sample it is also indicated in Fig. 2.

The change in the spectra due to 6-*O*-methylation can be a further support in the assignment. The two signals at 73.3 and 68.8 ppm which only appear in the methylated sample correspond to carbon atoms especially sensitive to the methylation. The signals from these carbon atoms in the parent species must decrease with increasing degree of methylation. The effect should be noticeable going from Fig. 2 to Fig. 3. Based on this argument the two carbon atoms (other than G6) sensitive to 6-*O*-methylation are assigned to the signals at 75.1 and 68.6 ppm in the non methylated sample. From the chemical shifts of the model molecules given in Table 2 these signals can be assigned to the G5 and the G4 carbons, respectively. The assignment of the signals at 73.3 and 68.8 ppm must then be G5′ and G4′, respectively. This assignment yields the conclusion that only the two ring carbons G5 and G4 closest to the position of methylation are sensitive to the methylation, the effect being −2.5 ppm and +0.2 ppm, respectively. When the unassigned signals are considered it is now possible to assign the G3, the G2 and the A2 carbons from the chemical shifts alone, as given in Table 1, column II. At this stage three carbons from the A-residue remain to be assigned the remaining signals at 79.9, 77.1 and 75.4 ppm, but further information is necessary before an unambiguous assignment can be made. The total assignment given above is summarized in column II of Table 1, and in Fig. 3.

SUPPLEMENTARY REMARKS

The spectra shown in Figs. 2 and 3 show large differences in peak heights also for carbons with the same *N* value. Measurements of spin lattice relaxation times ensure that the spectra shown correspond to fully relaxed samples. Additional experiments have so far shown that this phenomenon is due to an unexpected variation in relaxation time for the involved carbons. A detailed examination of the ^{13}C relaxation in agarose is in progress, together with examination of the effect of temperature and gelation on the ^{13}C NMR spectra.[15]

EXPERIMENTAL

Commercially available agarose (LITEX) was used for the experiments. The spectra were recorded using 5 % w/v solutions in D_2O-H_2O mixtures, with methanol as internal standard. The spectra shown correspond to the following sample: 200 mg agarose, 3 ml H_2O, 1 ml D_2O and 50 μl CH_3OH. The solution was made directly in the 10 mm NMR tube by heating the agarose–water mixture to 95–100 °C for 1/2–1 h.

The instrument used was a Bruker HX 270 spectrometer equipped with a Fourier transform system, and operating with quadrature detection at 67.9 MHz for ^{13}C NMR. The proton decoupled spectra were accumulated in 16 K data points using a spectral width of 17 240 Hz. The spectra shown in Figs. 2 and 3 correspond to 3600 scans. The repetition time was 2.5 s and a 90° RF puls of 12 μs was used. Gated and off resonance decoupling experiments were performed using 32K data points for the same spectral width. The spectrum shown in Fig. 4 corresponds to 5200 scans. Deuterium in the *solvent* was used for the field-frequency lock, and the ^{13}C chemical shifts are reported using methanol, as a secondary internal standard, at 49.3 ppm relative to TMS.

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