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Note

Carbon-13 nuclear magnetic resonance spectroscopy of covalently crosslinked dextran (Sephadex® G) hydrogels

RENÉ SCHERRER, ROBERT LUNDIN, MABRY BENSON, AND SUE C. WITT
Western Regional Research Center, Agricultural Research Service, United States Department of Agriculture, Berkeley, California 94710 (U.S.A.)

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Gels are elastic, solid-like coherent systems of continuous structure and composed of at least two components[1]. Polysaccharides form several different types of gel with water. Most commonly, long-chain polysaccharides produce hydrogels through complex junction-zones, thermoreversible helical regions, or other coaggregation phenomena[2,3]. However, more-stable hydrogels are obtained by covalently crosslinking polysaccharide chains. Sephadex® G (Pharmacia AB, Uppsala, Sweden) are such covalently crosslinked gels based on partially degraded fractions of dextran, a linear, (1→6)-linked α-D-glucan having little (1→3) branching[4]. They are made by mixing epichlorohydrin with dextran in concentrated sodium hydroxide solution; the resulting, three-dimensional network consists of 1-deoxyglycerol-1-yl ("glyceryl")-linked dextran chains having free glyceryl side chains[4] (1). The swelling and water content of the gels depend on the degree of crosslinking[1]. Crosslinking itself depends on the molecular weight of the dextran and on the relative proportions of epichlorohydrin to dextran during gelation[4]. For the Sephadex G gels, the water content increases from the most tightly crosslinked gel, G-10, having 1 g of water/g of dry gel through a series of gels (G-15, G-25, G-50, G-75, and G-100) of ever increasing water content to the most loosely crosslinked gel, G-200, which has a water content of 20 g of water/g of dry gel[4].

The structure, types and degrees of crosslinking, and physical properties of these Sephadex G gels are only partially known. This report describes gel characteristics obtained by liquid-state ^{13}C-n.m.r. spectroscopy. Liquid-state ^{13}C-n.m.r. spectroscopy, commonly used for analysis of polysaccharide structure[5], has been applied to covalently crosslinked synthetic gels[6,7], hydrogen-bonded polysaccharide hydrogels[3,8,9], and[10] Sephadex G-75. Solid-state, magic-angle spinning ^{13}C-n.m.r. spectroscopy has also been used for gels[11], although ultracentrifugal gel compression[12] could decrease the solvent content of the gel.

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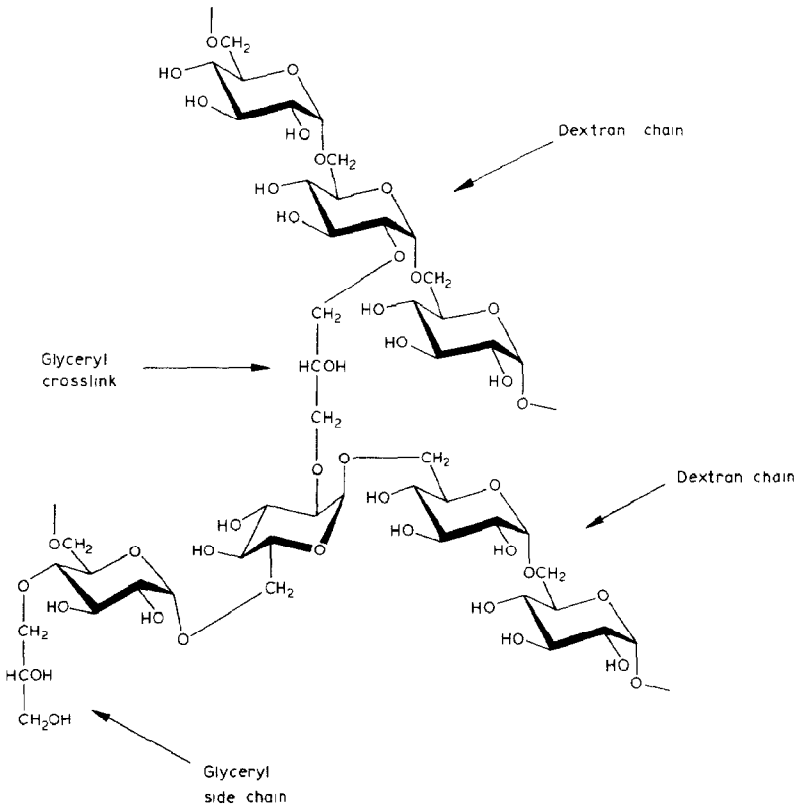

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EXPERIMENTAL

Natural abundance ^{13}C-n.m.r. spectroscopy was performed at 25.03 MHz with a JEOL PFT 100/EC 100 pulsed Fourier-transform spectrometer. Proton-noise-decoupled spectra (50,000–435,000 scans) were accumulated with a single 90° pulse and repetition rates of 0.52–4 s. Spectral width was usually 4 KHz, with 0.24–0.98 Hz nominal resolution. The primary internal reference was sodium 4,4-dimethyl-4-silapentanoate-*2,2,3,3-d*$_4$ (TSP), with acetonitrile (δ' 3.67 at 29°) as the secondary one. Spin–lattice relaxation times (T_1) were obtained by a fast inversion–recovery method[13], with an error estimate of ±15%. Line widths (full width at half maximum) were corrected for line broadening produced by apodization. Sephadex G gels and dextrans of number-average molecular weight ($\bar{M}_n$) of 1500, 3600, 5200, 21,000, and 123,000, and weight-average molecular weight ($\bar{M}_w$) of 2×10^6, all from Pharmacia, were analyzed in D_2O. Evenly packed suspensions of Sephadex G microbeads (wet-bead diameter 40–100 μm) were obtained by allowing previously deaerated, gelled suspensions to settle in 5-mm n.m.r. tubes at 29 and 90°. Resonances were identified from published spectra (Table I).

TABLE I

^{13}C-N.M.R. SPECTROSCOPY OF COVALENTLY CROSSLINKED DEXTRAN (SEPHADEX G) GELS IN D_2O

No.[a]	δ'[b]	*Resonance detected*[c] *in G-*							*Assignment*[e]	*References*
		200	*100*	*75*	*50*	*25*	*15*	*10*[d]		
1	100.5	+	+	+	+	+	+	–	Glc C-1 (1→6) dextran main chain	14–17
A	101.9	+	+	+	–	–	–	–	Glc C-1 (1→3) dextran branch	17
B	100.8	–	–	–	–	+	–	–	Glc C-1 (1→6) with O-3 substitution	17
C	98.5	–	–	+	+	+	–	–	Glc C-1 (1→6) with O-2 substitution	17
2	74.2	+	+	+	+	+	–	–	Glc C-2 (1→6)	14–17
E	81	–	–	–	+	+	+	+	Glc C-2 (1→6) with O-2 substitution	17
3	76.2	+	+	+	+	+	–	–	Glc C-3 (1→6)	14–17
D	83	–	–	–	+	+	+	+	Glc C-3 (1→6) with O-3 substitution	17
4	72.3	+	+	+	+	+	–	–	Glc C-4 (1→6)	14–17
F	79	–	–	+	+	+	+	+	Glc C-4 (1→6) with O-4 substitution	17
5	73.0	+	+	+	+	+	–	–	Glc C-5 (1→6)	14–17
6	68.3	+	+	+	+	+	+	+	Glc C-6 (1→6)	14–17
K	63.5	+	+	+	+	+	+	+	Glc C-6 non-reducing endgroups	14
3′	65.4	+	+	+	+	+	+	+	Glyceryl C-3 side chain[f,g,h]	18
G	74.7	0	0	0	0	+	0	0	Glyceryl C-1 side chain[f,g]	18
	73.4	–	–	–	+	+	–	–	Glyceryl C-2 side chain[g,i]	
L	60.9	–	–	+	+	+	–	–	$-CH_2O-$[f,j]	18
	20.8	0	–	–	–	+	+	+	$-CH_3$[i,j]	
H	73.0	–	–	–	–	–	+	+	Glyceryl C-2 + Glc C-5 (1→6) ??	
I	71.5	–	–	–	–	–	+	+	[j]	
J	70.5	–	–	–	+	–	+	+	[j]	

[a]See Fig. 1A. [b]Chemical shifts (δ') in p.p.m. from sodium 4,4-dimethyl-4-silapentanoate-*2,2′,3,3′-d*$_4$ at 29°. [c] + detected, – not detected or resolved, 0 not determined. [d]G-10 was analyzed at 90° only. [e]Glc, α-D-glucosyl residues. [f]Determined by multiplicity-separation method[18] in G-25 only. [g]Glyceryl residues identified from Sadtler carbon-13 n.m.r. spectrum no. 2269. [h]Glyceryl C-3 also identified by addition of glycerol to G-50. [i]Peaks not shown or identified in Fig. 1A. [j]Not assigned.

RESULTS AND DISCUSSION

^{13}C-N.m.r. spectra of all but the most tightly crosslinked Sephadex G gels are dominated by the six major resonances of (1→6)-linked α-D-glucosyl residues of dextran chains (Fig. 1A). In G-25, G-15, and G-10 however, glucosyl (Glc) lines are broad, unresolved or absent, and "glyceryl" lines gradually dominate the spectrum. This is best seen for the C-1 region of Glc (δ' 98–102) where the C-1 (1→6) lines of Glc are absent in G-10 at 90° or G-15 at 29°, appear only as a broad band in G-15 at 90°, and are still broadened in G-25 at 29°. Analysis of the C-1 areas for Glc shows a decrease of unsubstituted Glc C-1 (1→6) in favor of 2, 3, and 4 substituted Glc C-1 (1→6) resonances as the water content decreases. Lower water content also corresponds to increased peak intensity of the δ' 78–84 region, attributed[14,17] to C-2, C-3, and C-4 of *O*-substituted Glc. The intensity of glyceryl C-3 (δ' 65.4), the free end-group of glyceryl side-chains, increases qualitatively in parallel with that of the δ' 78–84 peaks, indicating that the observed *O*-substitution

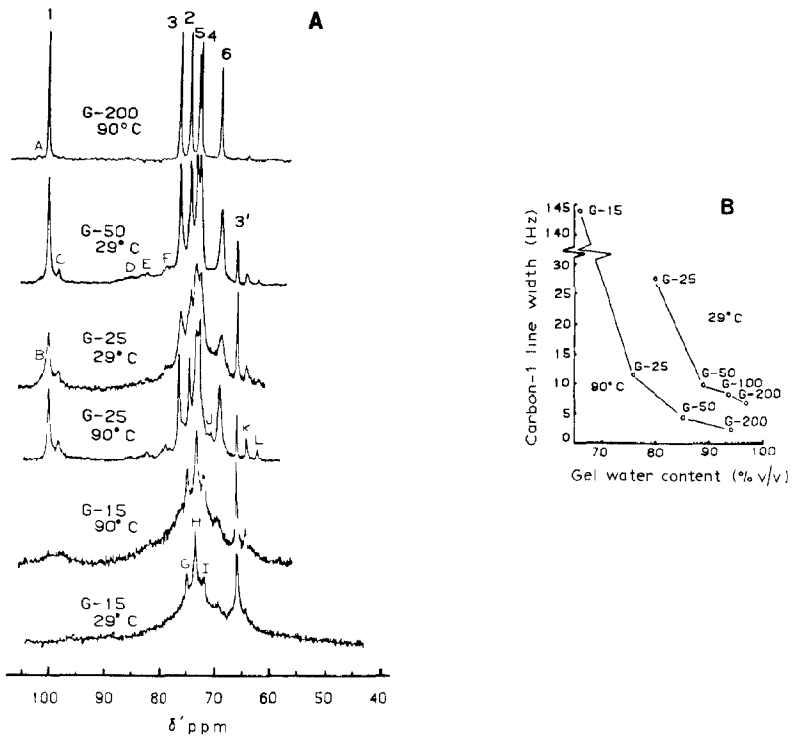

Fig. 1. ^{13}C-n.m.r. spectroscopy of covalently crosslinked dextran (Sephadex G) gels in D_2O. (*A*) Representative spectra. Chemical shift (δ') from sodium 4,4-dimethyl-4-silapentanoate-*2,2',3,3'-d*$_4$. Numbering and lettering correspond to Table I. (Glyceryl C-3 intensity, peak no. 3', of G-25 at 90° was decreased by saturation) (*B*) Line widths of (1→6)-linked α-D-glucosyl C-1 resonance of gels as a function of gel water (H_2O) content. Water content expressed as volume percent of total gel microbead volume.

of Glc residues arises mainly from glyceryl side-chains and not from crosslinks. Overlapping lines in the δ' 66–76 region, covering Glc C-2 (1→6) to C-6 (1→6), mask most glyceryl C-1 and C-2 lines. Glyceryl C-1 and C-3 could however be identified by virtue of their one-bond couplings to two protons by means of a multiplicity-separation method[18]. No evidence of glyceryl lines attributed solely to crosslinks was found. A preparation[4] of 100 (w/v)% dextran of $\bar{M}_n$ 21,000 in 3M sodium hydroxide with 33(v/v)% epichlorohydrin gave a tightly crosslinked gel that had broadened Glc peaks, an *O*-substituted Glc region, a glyceryl C-3 line, and few Glc C-1 (1→3) branches, being similar to Sephadex G gels.

Line widths of Glc resonances in gels are broader than those of carbon atoms of dextran solutions at the same weight concentration, for dextran molecular weights of 1500 to 2×10^6. They increase greatly with decreasing water content of the gel, as best seen for Glc C-1 (1→6) resonances (Fig. 1B). The spin–lattice relaxation-times (T_1) measured were 70–80 ms at 29° for Glc C-1 (1→6) in both Sephadex G-200 and G-25, and 150–160 ms at 29° for C-3 of glycerol in G-25 and G-15. The T_1 values of dextrans in solution do not vary appreciably over a wide

range of molecular weights: two dextrans of $\bar{M}_n$ 1500 and $\bar{M}_w 2 \times 10^6$ (both at a concentration of 32 g/L and at 29°) gave values 85–105 ms for Glc C-1 (1→6) and 50–60 ms for Glc C-6 (1→6). These dextran values are twice as high as published ones obtained at 20 MHz at a much higher concentration[10]. Lack of spectral resolution prevented determinations of T_1 of other gel carbon atoms.

The progressive broadening of all Glc gel resonances with increasing degree of crosslinking may be explained most reasonably on the basis of a gradual loss of mobility by all gel carbon atoms. However, the constant measured T_1 values of Glc C-1 (1→6) for both G-25 and G-200 also suggest that only a fraction of all gel carbon atoms give detectable, liquid-state n.m.r. signals, a phenomenon already described for partly immobilized gel-systems[6,7]. Quantitative ^{13}C-n.m.r. spectroscopy of the Sephadex G gels should indicate the fraction of immobilized carbon atoms. Line-shape analysis of accessible Glc residues, especially the Glc C-1 region, and structural determinations of crosslinks in gel fragments of smaller sizes, will give more complete information on the type and degree of *O*-substitution in Glc residues, both by crosslinks and glyceryl side chains. Line-shape and relaxation-time analyses should also give a dynamic picture of these gels.

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