Carbohydrate Research, 152 (1986) 279–282
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Structural determination of a water-insoluble glucan from *Streptococcus mutans* 6715 by carbon-13 nuclear magnetic resonance spectroscopy

HUGH M. DAVIS, WALTER J. BOYKO, AND JOHN R. EDWARDS
Villanova University, Chemistry Department, Villanova, PA 19085 (U.S.A.)
(Received September 18th, 1985; accepted for publication, December 2nd, 1985)

Dextrans, water-soluble polysaccharides containing mostly α-(1→6) linkages, are produced by both *Leuconostoc* and *Streptococcus* species. These polymers have been extensively studied by ^{13}C-n.m.r. spectroscopy[1,2]. In addition, many streptococcal species produce a water-insoluble glucan. The water-insoluble glucans comprise the matrix of plaque, and the organisms in plaque cause dental caries[3]. These glucans typically contain high proportions of α-(1→3) linkages in a linear arrangement, with α-(1→6) linkages either as branch points or extending linearly from branch points[4,5].

The structure of the water-insoluble glucan from *Streptococcus mutans* 6715 as determined by ^{13}C-n.m.r. is described in this paper. The ^{13}C-n.m.r. spectrum of the glucan (Fig. 1A) shows resonances at 102.4 and 100.7 p.p.m. for anomeric carbon atoms linked to O-3 and O-6, respectively. The values are in keeping with those reported by others[6,7] in their analyses of streptococcal glucans. Comparison of the areas of the resonances of the anomeric carbon atoms linked to O-3 and O-6 yield values of 69% α-(1→3) and 31% α-(1→6). These values correspond well with the chemical data based on periodate oxidation and methylation analysis reported by Davis *et al.*[4].

The resonances at 75.0, 73.6, and 73.1 p.p.m. are assigned to C-5, C-2 and C-4, respectively. These assignments were based on the data of Colson *et al.*[8] for nigerose. In addition, resonances for C-6 of α-(1→6)-linked residues (68.9 p.p.m.), for free exocyclic C-6 (63.8 p.p.m.), for C-3 of α-(1→3)-linked residues (84.6 p.p.m.), and for non-linked C-3 (76.5 p.p.m.) were noted in the spectrum.

The ^{13}C-n.m.r. spectrum of the glucan[7] from OMZ-176 shows a resonance at 102.8 p.p.m. due to α-(1→3) branch linkages. This resonance was not found in the spectrum of the native glucan, even though the polysaccharide is reported as containing 14% branching residues[4]. The large line-width in this spectrum was considered to be the major reason for not distinguishing between linear, non-branched α-(1→3) residues and branched residues linked α-(1→3).

The glucan was then subjected to an endodextranase that had been shown to hydrolyze α-(1→6) linkages in streptococcal glucans[9]. The ^{13}C-n.m.r. spectrum of

0008-6215/86/$ 03.50

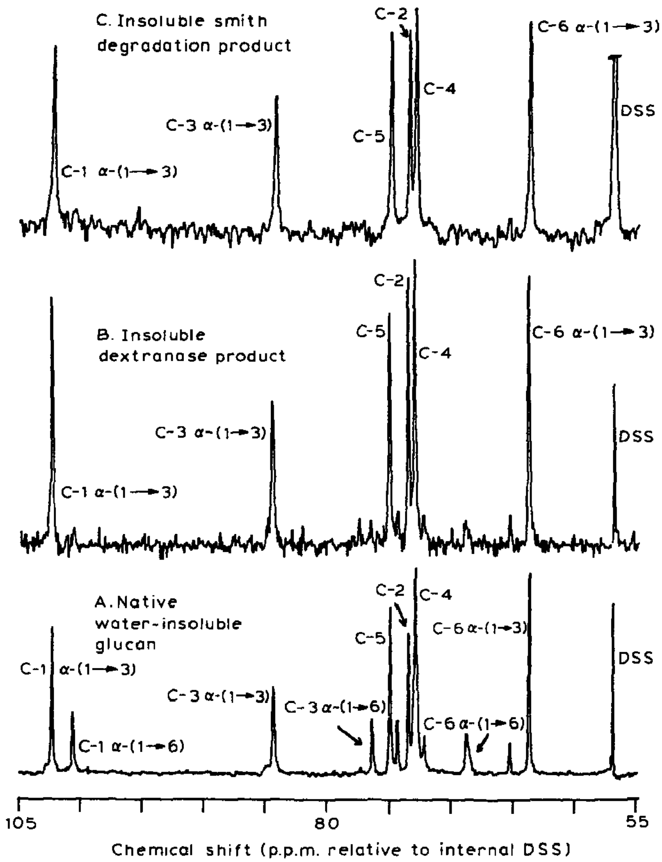


Fig. 1. ^{13}C-N.m.r. spectra at 50.3 MHz of the native water-insoluble (A), of the dextranase-treated (B), and of the Smith-degraded (C) glucans from *Streptococcus mutans* 6715 (200 mg, pD 14.1, 85°). Approximately 220,000 transients were taken for B and C, and 180,000 transients were taken for A.

the insoluble material remaining after treatment with dextranase (Fig. 1B and Table I) showed only minor resonances for C-1 involved in α-(1→6) linkages, for C-6 involved in a linkage, and for non-linked C-3. It was not possible to integrate anomeric resonances as the low-intensity peaks were masked by high noise because of low sample amounts. It was thought that the residue linked α-(1→6) to the main α-(1→3)-linked chain would, because of steric hindrance, remain intact following dextranase digestion. In fact, 74% of the original mass of the glucan remained insoluble after a 48-h incubation period, as compared to 69% following Smith degradation[4].

The ^{13}C-n.m.r. spectrum of the water-insoluble polysaccharide isolated following periodate oxidation and Smith degradation was recorded (Fig. 1C and Table I). The spectrum of the glucan following Smith degradation showed only those resonances for C-1 involved in an α-(1→3) glucosidic linkage, *O*-linked C-3, C-5, C-2, C-4, and free exocyclic C-6. This confirms that the α-(1→3) linkages are

TABLE I

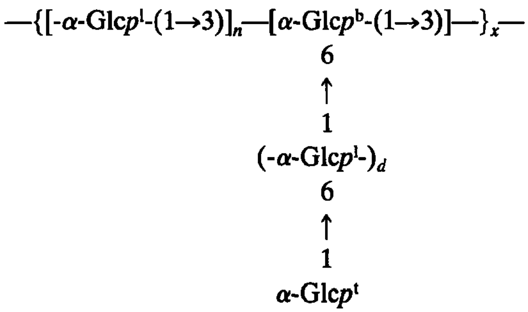
CHEMICAL SHIFTS[a] FROM ^{13}C-N.M.R. SPECTRA AT 50.3 MHz, 85°, AND pD 14, OF THE NATIVE WATER-INSOLUBLE GLUCAN (A), THE DEXTRANASE TREATED, WATER-INSOLUBLE GLUCAN (B), AND THE SMITH DEGRADED, WATER-INSOLUBLE GLUCAN (C)

Assignment	Streptococcus mutans *6715 Glucan*		
	A. Native	*B. Dextranase treated*	*C. Smith degraded*
C-1 α-(1→3)	102.4	102.4	102.4
C-1 α-(1→6)	100.7		
C-3 α-(1→3)	84.6	84.5	84.7
C-3 α-(1→6)	76.5		
C-5	75.0	75.1	75.1
C-2	73.6	73.6	73.6
C-4	73.0	73.1	73.1
C-6 α-(1→6)	68.9		
C-6 α-(1→3)	63.8	63.9	63.8

[a] Chemical shifts are in p.p.m. relative to internal DSS. The figure legends give experimental conditions and instrumental parameters.

contiguous in a linear sequence and that the α-(1→6)-linked residues extend from this main chain. Furthermore, it had been shown that the water-soluble fraction following Smith degradation gave rise to glycerol, and the soluble fraction from endodextranase digestion gave rise to glucose and isomaltose[4]. This result indicates that all of the α-(1→6) linkages are involved as side chains.

The *S. mutans* 6715 glucan is proposed to have the following chemical structure:



$$\begin{array}{c} -\{[-\alpha\text{-Glc}p^{l}\text{-}(1\rightarrow3)]_n-[\alpha\text{-Glc}p^{b}\text{-}(1\rightarrow3)]-\}_x- \\ 6 \\ \uparrow \\ 1 \\ (-\alpha\text{-Glc}p^{l}\text{-})_d \\ 6 \\ \uparrow \\ 1 \\ \alpha\text{-Glc}p^{t} \end{array}$$

where α-Glc*p* indicates an α-D-glucopyranosyl residue; the superscripts l, b, and t indicate linear residues, branching residues, and terminal residues, respectively; and the subscripts *n, d* and *x* indicate lengths of the sequence in the polymer. The ^{13}C-n.m.r. analysis of the native glucan, of the insoluble material from dextranase digestion, and of the insoluble material following periodate oxidation and Smith degradation confirms the postulated structure based on methylation analysis and periodate oxidation[4].

EXPERIMENTAL

Isolation and purification of water-insoluble glucan. — The water-insoluble glucan was produced by incubation of the crude concentrate of D-glucosyltransferases isolated from *Streptococcus mutans* 6715 (F. P. Mertz of Eli Lilly and Co., Indianapolis, IN) with sucrose (80mM) in a phosphate–KCl–Tween 80 buffer (50mM phosphate, M KCl, 0.05% Tween 80) according to Figures and Edwards[10].

Digestion by dextranase. — Dextranase digestion of the native glucan was accomplished by suspending the glucan samples (300 mg) in acetate buffer (18 mL, 50mM, pH 5.1) containing dextranase (0.30 mL, 1700 units/mg, Miles Scientific, Naperville, IL) from *Chaetomium gracile*. Insoluble material remaining after treatment with dextranase was isolated by centrifugation, washed, and analyzed by ^{13}C-n.m.r. spectroscopy.

Periodate oxidation and Smith degradation. — Periodate oxidation and Smith degradation of the polysaccharide has been previously published[4]. Following Smith degradation, the insoluble material remaining was centrifuged, washed, and analyzed by ^{13}C-n.m.r. spectroscopy.

Carbon-13 nuclear magnetic resonance spectroscopy. — ^{13}C-Fourier-transform n.m.r. spectra were obtained at 50.3 MHz with a Varian XL-200 spectrometer. Carbohydrate (300 mg) was suspended in D_2O (3 mL). Powdered sodium hydroxide (60 mg) and sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS, 90 mg, Aldrich Chemical CO., Milwaukee, WI) were added. The concentration of NaOH was 0.5M with a pD of 14.1 (pH plus 0.4). All spectra were obtained at 85°. An acquisition time of 0.3 s (3–4 times T_1) with no delay (corresponding to 6840 points) and a 90° pulse-angle (13.4 μs) was used. Typically, 180,000–250,000 transients were needed for suitable spectra. All spectra were broad-band proton-decoupled. Chemical shifts are expressed as p.p.m. relative to internal DSS.

ACKNOWLEDGMENT

The authors thank Villanova University for support of this research.

REFERENCES

1 F. R. Seymour, R. D. Knapp, E. C. M. Chen, S. H. Bishop, and A. Jeanes, *Carbohydr. Res.*, 74 (1979) 41–62.
2 F. R. Seymour, R. D. Knapp, and B. L. Lamberts, *Carbohydr. Res.*, 84 (1980) 187–195.
3 J. D. DeStoppelaar, K. G. Konig, A. J. M. Plasschaert, and J. S. VanDerHoeven, *Arch. Oral Biol.*, 16 (1971) 971–975.
4 H. M. Davis, H. B. Hines, and J. R. Edwards, *Carbohydr. Res.*, in press.
5 S. Shibata, I. J. Goldstein, and J. J. Kirkland, *Carbohydr. Res.*, 120 (1983) 77–84.
6 T. Usui, H. Sugiyama, S. Seto, S. Araya, T. Nisizawa, S. Imai, and K. Kosaka, *J. Biochem. (Tokyo)*, 78 (1975) 225–227.
7 P. Colson, H. C. Jarrell, B. L. Lamberts, and I. C. P. Smith, *Carbohydr. Res.*, 71 (1979) 265–272.
8 P. Colson, H. J. Jennings, and I. C. P. Smith, *J. Am. Chem. Soc.*, 96 (1974) 8081–8087.
9 New Product Announcement, Miles Scientific, Naperville, IL.
10 W. R. Figures and J. R. Edwards, *Carbohydr. Res.*, 79 (1979) 245–253.