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Note

Structural determination of D-fructans from *Streptococcus mutans*, serotype *b*, *c*, *e*, and *f* strains, by ^{13}C-n.m.r. spectroscopy

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Streptococcus mutans secretes two or three kinds of D-glucosyltransferase and, in serotype *b*, *c*, *e*, and *f* strains, D-fructosyltransferase[1]. These enzymes cooperatively serve to produce, from sucrose, adhesive, water-insoluble polysaccharides that induce dental caries. D-Glucans produced by the D-glucosyltransferases have been extensively studied by gas–liquid chromatography (g.l.c.)[1]. However, only one[2] chemical analysis of the structure of *S. mutans* D-fructan has as

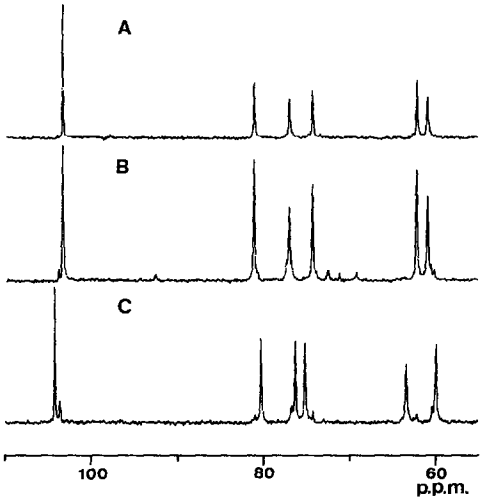

Fig. 1. ^{13}C-N.m.r. spectra of *S. mutans* LM7 fructan (A), inulin (B), and levan (C) in D_2O at 21°. [Spectra were recorded with an acquisition time of 1.0 s, a pulse angle of 45°, a digital resolution of 1.0 Hz, and transients of 20,000, 30,000, and 29,400 for A, B, and C, respectively. Chemical shifts are expressed as p.p.m. relative to external tetramethylsilane.]

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TABLE I

CHEMICAL SHIFTS[a] FOR ^{13}C-N.M.R. SPECTRA OF D-FRUCTANS

Carbon atom	S. mutans D-*fructans*					
	BHT (b)	*Ingbritt (c)*	*LM7 (e)*	*OMZ175 (f)*	*Inulin*[b]	*Levan*[b]
C-1	60.9	60.9	60.9	60.9	60.9	59.9
C-2	103.2	103.2	103.3	103.3	103.3	104.2
C-3	77.0	77.0	77.0	77.0	77.0	76.3
C-4	74.3	74.3	74.3	74.3	74.3	75.2
C-5	81.1	81.1	81.1	81.1	81.1	80.3
C-6	62.2	62.1	62.2	62.1	62.2	63.4

[a]In p.p.m. from external tetramethylsilane. [b]Assignment cited from ref. 3.

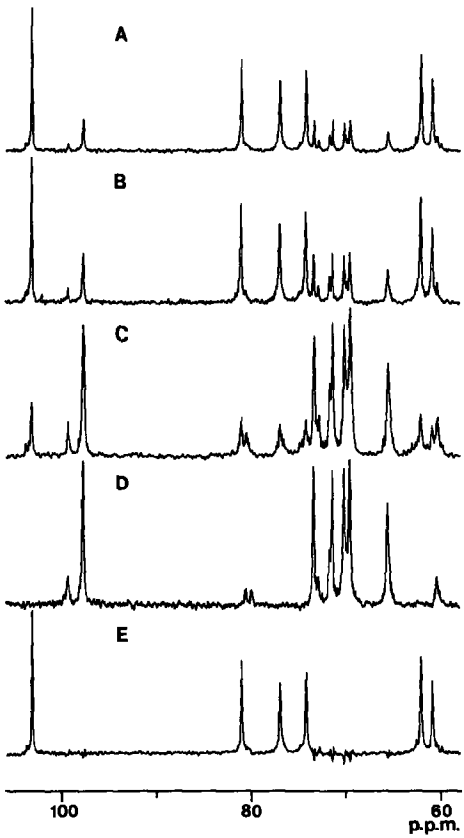

Fig. 2. ^{13}C-N.m.r. spectra of *S. mutans* polysaccharides in D_2O at 21°. [A, Ingbritt (23,400 transients); B, OMZ175 (29,000 transients); C, BHT (30,000 transients); D, 6715 glucan (30,000 transients); E, difference spectrum of the Ingbritt spectrum (A) subtracted by 6715 spectrum with 0.18-fold intensity of the original (D). Chemical shifts are expressed as p.p.m. relative to external tetramethylsilane.]

yet been reported, mainly because D-fructofuranosides are heat-labile and partly decompose while being converted into derivatives suitable for analysis by g.l.c. We now report the structures of D-fructans from *S. mutans*, serotype *b*, *c*, *e*, and *f* strains, that were explicitly determined by ^{13}C-n.m.r. spectroscopy.

The D-fructan produced by purified D-fructosyltransferase from *S. mutans* LM7 (serotype *e*) showed six resonances, at 103.3, 81.1, 77.0, 74.3, 62.2, and 60.9 p.p.m. (see Fig. 1A). These chemical shifts coincide exactly with those respectively assigned to C-2, C-5, C-3, C-4, C-6, and C-1 of inulin, and definitely not with those of levan[3] (see Fig. 1B and C, and Table I), indicating that *S. mutans* LM7 D-fructan is of the inulin type and contains linear, β-(2→1) linkages.

S. mutans BHT (*b*), Ingbritt (*c*), and OMZ175 (*f*) D-fructosyltransferases were obtained as partially purified preparations from each culture supernatant liquor by specifically removing α-(1→3)-D-glucan synthase[4] by passage through an immunoabsorbent column. The polysaccharides produced by each preparation exhibited the six resonances identical with those for C-1 to C-6 involved in β-(2→1)-D-fructofuranosyl residues (see Fig. 2A, B, and C, and Table I). Additional resonances were observed at 99.3, 97.7, 80.6, 73.4, 72.9, 71.7, 71.4, 70.2, 69.6, 65.6, and 60.5 p.p.m., which are in the spectral regions for α-D-glucopyranosyl residues[5,6] and correspond to those of *S. mutans* 6715 α-D-glucan, which is composed of 87% of (1→6) and 6% of (1→3) linkages, and 7% of (1→3),(1→6)-linked branches[7] (see Fig. 2D). Obviously, these resonances were of D-glucans produced by one or two D-glucosyltransferases coexisting in each of the D-fructosyltransferase preparations, because the D-glucosyltransferases from Ingbritt (*c*), OMZ175 (*f*), and probably from BHT (*b*)[1], were catalytically similar to that from 6715 (ref. 7) used here. The difference spectrum (see Fig. 2E), which was recorded by subtracting the spectrum of 6715 glucan (see Fig. 2D) lowered to 0.18 in intensity from the spectrum of Ingbritt polysaccharide (see Fig. 2A), resembled the spectrum of the pure D-fructan from LM7 (see Fig. 1A). These observations indicated that *S. mutans* serotype *b*, *c*, and *f* D-fructans are also connected by β-(2→1) linkages.

We concluded that, in spite of serological differences among the strains used, *S. mutans* D-fructans are composed of β-(2→1)-D-fructofuranosyl residues. The results of the present study agree with the previous report[2] that the D-fructans produced by crude enzymes from *S. mutans* serotype *b* and *c* strains mainly contain β-(2→1) linkages, as analyzed by g.l.c.–mass spectroscopy; but the resonances corresponding to the branch observed in the report[2] were not detected in the present study by ^{13}C-n.m.r. analysis. In addition, a minor resonance at 103.8 p.p.m. in the anomeric region appeared in the spectra of inulin (see Fig. 1B) and BHT D-fructan (see Fig. 2C); this resonance may arise from C-2 atoms of D-fructofuranosides linked to the adjacent D-glucosyl residues, as found, in deuterium oxide[8], in β-(2→1)-D-fructosyl oligomers, including one terminal D-glucose unit.

EXPERIMENTAL

Purification of enzyme. — *Streptococcus mutans* LM7 (serotype *e*) D-fructosyltransferase was purified from cell-free, culture supernatant liquor by precipitating with 1.22 vol. of ethanol, and chromatofocusing, and was electrophoretically homogeneous, with an isoelectric point[1] of pH 4.5. The enzyme from *S. mutans* BHT (*b*), Ingbritt (*c*) (ref. 9) or OMZ175 (*f*) was partially purified from the ethanol precipitate by passing through an affinity column of Protein A–Sepharose CL-4B gel linked with antibody to the α-(1→3)-D-glucan synthase purified from[4] Ingbritt (*c*). In an isoelectrofocused gel[10], these preparations exhibited the activities synthesizing D-fructan and water-soluble D-glucan, but not α-(1→3)-D-glucan. *S. mutans* 6715 (*g*) D-glucosyltransferase (isoelectric point, pH 5.5), synthesizing α-(1→6)-D-glucan having 6% of α-(1→3) linkage and 7% of (1→3),(1→6)-linked branch[7], was purified by means of a DEAE-Sepharose column[1], and was immunologically and electrophoretically homogeneous.

Synthesis of polysaccharide. — The purified, or partially purified, enzymes were incubated with 5% sucrose in 0.1M sodium phosphate, pH 6.5, containing 0.01% of merthiolate. The 6715 glucan and the polysaccharides of the other strains were collected as 75% and 80% ethanol precipitates, respectively, and washed three times by suspending in 0.1M NaCl and precipitating with ethanol. The 6715 D-glucan and the LM7 D-fructan were not contaminated with D-fructan and D-glucan, respectively, as judged by the cold anthrone method[11] and the phenol–sulfuric acid method[12]. The BHT, Ingbritt, and OMZ175 polysaccharides were composed of D-fructan and D-glucan, and the D-fructan contents were 25, 72, and 66%, respectively. The LM7 fructan swelled in D_2O, and formed a smoothly dispersed, opalescent solution within 15 min at 60°, the BHT, Ingbritt, and OMZ175 polysaccharides behaved similarly. The 6715 D-glucan in D_2O also became opalescent, although it was more viscous than the LM7 D-fructan.

N.m.r. spectroscopy. — ^{13}C-N.m.r. spectra were recorded at 67.8 MHz with a JEOL GX-270 spectrometer in the pulsed, Fourier-transform mode with complete proton-decoupling at 21°. Levan of *Aerobacter levanicum* and inulin were purchased from Sigma and Tokyo Chemical Industry, respectively. Polysaccharides (26–60 mg) were suspended in D_2O (0.6 mL), heated for 15 min at 60°, and poured into 5-mm outside tubes. Samples thoroughly dissolved in 0.5M NaOD were also used, in order to examine whether additional resonances, or relative changes in intensity of resonances, or both, would or would not appear. The resultant spectra were essentially equivalent to those in D_2O, although most of the resonances in the alkaline solutions shifted slightly downfield, and the resonances for C-1 (99.3 p.p.m.) and C-3 (80.6 p.p.m.) of D-glucans shifted to 101.9 and 84.4 p.p.m., respectively. Chemical shifts are expressed as p.p.m. relative to external tetramethylsilane.

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