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STRUCTURAL DETERMINATION OF WATER-SOLUBLE DEXTRAN BY C-13 AND PROTON NMR SPECTROSCOPY

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Our preliminary study on glucobioses showed that C-13 NMR spectroscopy is a useful tool for the determination of anomeric configuration of glycosidic linkage (1). This report describes that the combined use of C-13 and proton NMR spectroscopy is also very useful for the structural determination of water-soluble glucans without using chemical modification or degradation. It has been reported that a water-soluble dextran obtained from Leuconostoc mensenteroides NRRL B-1299 has about a 59% of α-1,6-, a 34% of α-1,2- and a 7% of α-1,3-linkage determined by the methylation method (2,3). We selected this dextran as an example.

The proton-noise-decoupled C-13 FT spectrum of this dextran in heavy water showed three peaks due to α-linked anomeric carbons between 99.4 and 97.2 ppm and three peaks due to C-6 carbons between 67.6 and 62.3 ppm from an external TMS standard as shown in Fig. 1 (4,5). The chemical shift of peak 15 at 62.3 ppm is identical with that of the non-substituted C-6 carbon of glucose and that of peak 13 at 67.6 ppm with that of the C-6 carbon involved in the glycosidic linkage of isomaltose (α-1,6) and of clinical dextran (α-1,6 linear polymer). The chemical shift of peak 1 at 99.4 ppm is identical with that of the anomeric carbon involved in the α-1,6-linkage, and peak 3 at 97.2 ppm can be assigned to the anomeric carbon involved in the α-1,2-linkage. Peak 14 at

64.9 ppm should be assigned to the C-6 carbon bonded to the anomeric oxygen of the glucose unit, which has the C-2 carbon involved in the α-1,2-linkage, because α-kojibiose (α-1,2) has the same chemical shift tendency due to 1,2-steric interaction (5). From the same reason, peak 2 at 98.0 ppm could be attributed to the anomeric carbon of the α-1,6-linkage with the α-1,2-inter-chain linkage.

Since the same-numbered carbons in glucans are in similar environments and have similar chemical shifts, we can assume that the ratio of the change of the intensities of the same-numbered carbons due to the change of experimental condition (e.g. H_1, H_2 power, repetition time and so on) is equal (6). Hence, the above discussion may be confirmed by comparison of the signal intensities. The intensity ratio of peaks 1, 2 and 3 was 41:33:26, respectively, and that of peaks 13, 14 and 15 was 39:24:37, respectively. As a result, the ratio of the C-6-substituted glucose and the non-C-6-substituted one is 67:33 or 63:37, and these values are almost equal to those from the methylation result (2,3). Also, we can confirm that peaks 3 and 14 were attributed to carbons suffering the same kind of steric interaction, because their contents are similar. Peak 4 at 83.0 ppm and peak 5 at 77.7 ppm should be assigned to the C-3 and C-2 resonances involved in the α-1,3- (80.3 ppm) and the α-1,2-linkage (77.1 ppm), respectively, from the consideration of the data on glucobioses and trioses. The anomeric signal of the α-1,3-linkage might be hidden under noise signals under the present experimental condition.

The proton spectrum of the dextran in D_2O also provided much information. The anomeric signals appeared at the lowest field between 5.30 and 4.95 ppm from an internal DSS standard and were easily distiguished from other proton signals as shown in Fig. 2. Signals A, B+C and D should be assigned to the anomeric protons of the α-1,3-, the α-1,2- and the α-1,6-linkage, respectively, since the anomeric proton signals of the corresponding glucobioses and trioses have the very similar chemical shifts and coupling constants (7). The intensity ratio of signals A, B+C and D is 8:52:40, respectively. However, signals B and C have almost equal intensities. Peaks 2 and 3 in the C-13 spectrum were very close to each other, and the chemical shift of a proton behaves the reverse way

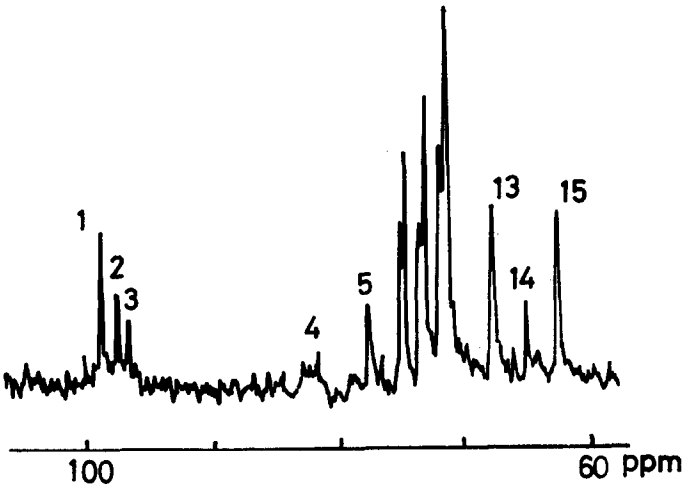

Fig. 1. The proton-noise-decoupled C-13 FT NMR spectrum of the dextran at 25.1 MHz. Saturated solution at room temp. Solvent; D_2O. Internal D lock. Pulse width; 12μsec. Repetition; 2.5sec. Shift error; ±0.1ppm. JEOL PFT-100 spectrometer and EC-6 computor system.

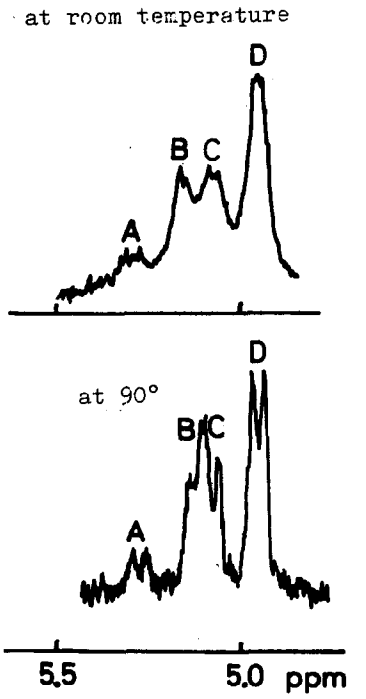

Fig. 2. The proton NMR spectrum of the dextran at 100 MHz. Saturated soln. in D_2O. Scanning rate; 108 ppm/250sec. Lock on internal DSS. JEOL FS-100 spectrometer.

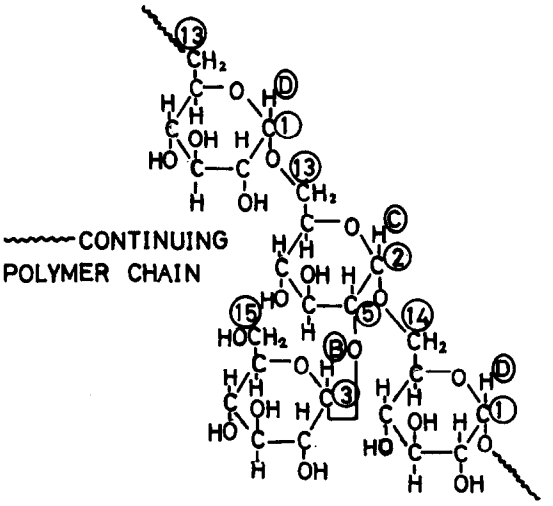

Fig. 3. The partial structure of the dextran. The numbering in this figure corresponds to the signal numbering of the spectra.

of that of the carbon attached for the bond polarization of sugar due to steric interaction (8). It can be assumed that one of signals B and C is assigned to the anomeric proton involved in the α-1,6-linkage with the α-1,2-interchain linkage and that the other is attributed to the one involved in the α-1,2-linkage. As a result, the ratio of the signal intensities is well interpreted and is consistent with the result from the C-13 spectrum.

The temperature dependence of the proton spectrum is also interesting. The signals became sharper at 90° than at room temperature as shown in Fig. 2. This change was essentially reversible. We can assume the amount of the non-reducing end unit from that of the non-C-6-substituted unit. Hence, the length of the branching units involved in the α-1,2-linkage might be relatively short, and the structure of the dextran might be like a comb. The teeth of this comb-like structure might gear into each other at room temperature and might be free at 90°. Signal B can be assigned to the anomeric proton involved in the α-1,2-linkage, since the short branching chain can move more easily than the long main chain. Thus, this structural assumption well explains the temperature dependence of the spectrum.

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