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Dextran structural factors which affect binding to the lectin of *Streptococcus cricetus*

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Oral bacteria, such as *Streptococcus cricetus* and *S. sobrinus*, are aggregated by high molecular weight fractions of the linear α-(1→6) dextran produced by *Leuconostoc mesenteroides*. The linkage specificity of this interaction with a cell surface lectin of the streptococci is high. As a further probe of factors affecting recognition, we prepared partially methylated samples of dextran of average MW of 2×10^6 and 10^4. ^{13}C NMR spectroscopy proved to be a convenient method for determining both site and degree of monomethylation and dimethylation of the polymer: relative reactivity of the OH groups was established as O2: O4: O3 = 4.3: 1.7: 1.0. A methylation DS of only 0.17 caused almost complete loss of recognition by the lectin. A structural feature of dextran oligomers is suggested to explain this result. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Bacteria use specific surface structures, and host or bacterium-derived molecules, frequently oligosaccharides, to attach themselves, with selectivity, to substrata (Ofek & Doyle, 1994). Oral streptococci employ sucrose to synthesize hydrophilic α-(1→6) and hydrophobic α-(1→3) linked glucans, and these polysaccharides are constituents of cariogenic plaque. The factors that govern the biosynthesis of these glucans have been ascertained under controlled growth conditions (Walker *et al.*, 1990; Cheetham *et al.*, 1991). Further, both polymers are required for the adhesion of the oral bacteria to smooth surfaces (Hamada, 1983). Several of these streptococci have evolved surface proteins (lectins) which are capable of complexing with monosaccharides or oligosaccharides. The lectins probably play a role in the adhesion of the bacteria to surfaces, and may be virulence factors in promoting the colonization of the organisms on the surfaces of teeth. High molecular weight fractions of the linear α-(1→6) linked glucan, known as dextran, produced by *Leuconostoc mesenteroides* strains are capable of agglutinating several varieties of oral streptococci; this suggests that the native α-(1→6) linked glucans are the molecular receptors of the bacterial adhesins in dental plaque (Doyle & Taylor, 1994; Drake *et al.*, 1988; Landale *et al.*, 1987). To probe structural factors affecting recognition of dextran by the glucan-binding lectin of *Streptococcus cricetus* AHT and *S. sobrinus* 6715 we prepared samples of dextran (average MW of 2×10^6 and 10^4) having low degrees of methylation. Herein we briefly describe the methylation procedure, and the use of ^{13}C NMR spectroscopy for the determination of the sites of methylation. Finally, we propose a structural explanation for the strong inverse effect of methylation on binding of dextran by the lectin of *S. cricetus*.

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EXPERIMENTAL

General

Melting points are uncorrected. Microanalyses were performed by Midwest Microlab, Indianapolis, Indiana. TLC was performed on silica gel 60 F-254 (Alltech) plates. Compounds were made visual by UV light and/or by charring with 10% H_2SO_4. Column chromatography was carried out with silica gel 60 (Merck, 70-230 mesh). Optical rotations were determined using a Jasco Model DIP-370 digital polarimeter. Evaporation was carried out under reduced pressure at 40–50°C.

^{13}C NMR spectra were recorded on a Bruker AMX500 FT-NMR spectrometer. For signal assignments on low MW dextran (Fluka 31394, $M_r \sim 1500$), 1-D HOHAHA experiments (Kessler *et al.*, 1986) were used to obtain subspectra of the reducing terminus residue by selective excitation (60 ms Gaussian pulse) of the

resolved anomeric protons. Polarization transfer to the ring protons was accomplished with MLEV-17 sequences (Bax & Davis, 1985) for 100 ms. 2-D HOHAHA-COSY experiments (Homans, 1990) gave the correlation pattern for the reducing terminus rings. These experiments use the 1D-HOHAHA sequence as the excitation pulse input to a COSY experiment. Phase-sensitive detection with TPPI was used (Marion & Wuthrich, 1983). C-H correlation spectra were used to confirm ^{13}C signal assignments. ^{13}C NMR spectra of methylation products were ^{1}H- decoupled. Acquisition time was 0.9 s, and d_1-delay time 5 s, to allow for complete relaxation so that quantitative results could be obtained by integration. For integration, the anomeric C signals were used as internal reference, and the $COCH_3$ signals between δ82–85.7 integrated to determine DS. The sample concentrations were 80 mg of methylated dextran in 0.5 mL of D_2O.

MM2 calculations were done using MacMimic/MM2(91) software available from InStar Software, Odeon Research Park, S-223 70 Lund, Sweden. Dextran triads and tetrads were modeled at $\varepsilon = 80$ from minimized methyl α-D-glucopyranosides having the secondary OH groups in the r' conformation (Dowd *et al.*, 1992). In the minimized triads ϕ and ψ_{6R} angles had the following ranges (ω, ϕ, ψ_{6R}): residues linked gg: $-63\pm2°$, $-36\pm2°$, $-38\pm4°$; residues linked gt: $62\pm1°$, $-45\pm2°$, $50\pm8°$ (ω: O5-C5-C6-O6; ϕ: H1-C1-O1-C6'; ψ: C1-O1-C6'-H6'*R*). The triad having the gt→gg angle ω sequence had a calculated steric energy at least 3.5 kcal lower than other angle ω combinations. Steric energies for triads bearing OCH_3 groups at positions 2 or 4 (arrows, Fig. 1) were raised 5.3 or 7.3 kcal, respectively.

General Methylation Procedure

Sodium hydride (97% dry) as limiting reagent was weighed into a dry three necked flask and kept under dry N_2 gas. It was then mixed with 10 mL of dry DMSO (freshly distilled over CaH_2 under high vacuum). The contents were heated at 50–60°C with constant stirring (magnetic stirrer) for 1 h or, until a yellow greenish color was observed in the solution. Then, 1.0 g of dextran (Sigma D-5376), MW $\sim 2 \times 10^6$, or Sigma D-9260, MW $\sim 10^4$, or equivalent, having less than 3% branching as estimated by NMR spectroscopy, predried under vacuum at 100°C for 2 h), dissolved under N_2 in 20 mL of dry DMSO, was transferred into the flask containing the methylsulfinylmethyl anion. The mixture was stirred for 4 h at rt before adding a 1.5 molar excess of redistilled iodomethane. Stirring was continued for 6 h to effect complete methylation. Mixed resin (Dowex MR-3), 3–5 g, was then added to the reaction mixture. After stirring for 30 min, the mixture was filtered and concentrated at less than 1 mm Hg. The residue was triturated with methanol; the resulting solid product was separated by filtration and washed twice with 50 mL of methanol. Finally it was dried under high vacuum over P_4O_{10}. The dried samples were subjected to NMR spectral analysis. The methylation results follow: (dextran sample: mmol NaH/g dextran (total DS)) MW 10^4 no.1: 0.38 (0.03); no.2: 3.08 (0.15); no.3: 3.79 (0.19); MW 2×10^6 no.1: 2.62 (0.15); no.2: 3.42 (0.17); no.3: 4.13 (0.34); no.4: 6.75 (0.29); no.5: 15.25 (0.72).

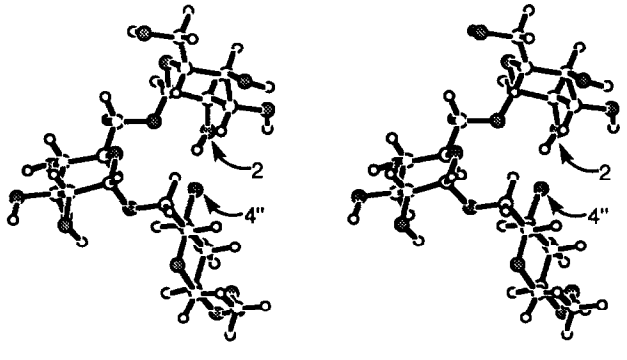


Fig. 1. A stereo view of an MM2-minimized molecular model of a dextran triad. The conformations about angle ω , from the non-reducing residue at top, are gt, gg. The arrows indicate the sites of conformation-destabilizing methylation at O2 and O4". The minimized structure suggests the presence of an OH2–O4" H bond.

Methyl 6-*O*-methyl-α-D-glucopyranoside, 1a

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-methyl-α-D-glucopyranoside (Liptak *et al.*, 1975), 5.0 g, was dissolved in 200 mL of 97% ethanol, and 9 g of metallic Na was added rapidly in small portions. After 1 h stirring the reaction mixture was warmed to complete reaction the of Na. H_2O (200 mL) was added to get a clear solution. The clear solution was neutralized by passing over a column of Amberlist 15 sulfonic acid resin. Evaporation of the eluate gave a syrup. The syrup was purified by column chromatography (ethyl acetate:methanol, 8:1, $R_f = 0.3$) to give a syrup: yield 1.51 g (70%); $[\alpha]_{20}$ 128° (c 0.5; H_2O); lit (Helferich *et al.* 1926) $[\alpha]_{20}$ 127.9°; ^{1}H NMR (D_2O) δ4.58 (d, 1H, $J_{1,2} = 3.45$ Hz, H1), 3.50–3.55 (m, 2H, overlapped resonances of H5 and H6a), 3.48–3.43 (m, 2H, overlapped resonances of H6b and H3), 3.34 (2d, 1H, $J_{2,1} = 3.45$ Hz, H2), 3.21 (s, 3H, $1OCH_3$), 3.19 (s, 3H, $6OCH_3$), 3.15 (dd, 1H, H4).

Methyl 2,6-di-*O*-methyl-α-D-glucopyranoside, 1b

Methyl 3,4-di-*O*-benzyl-2,6-di-*O*-methyl-α-D-glucopyranoside (Helferich *et al.*, 1926) (2.0 g) was debenzylated using the procedure for **1a**. The resulting syrup was purified by column chromatography ($CHCl_3$: CH_3OH: 9:1, $R_f = 0.35$) to yield 0.71 g (65%) of a syrup; $[\alpha]_{20}$ 137.7° (0.597 g, $CHCl_3$); ^{1}H NMR (D_2O) δ4.80 (d, 1H, $J_{1,2} = 3.5$ Hz, H1), 3.51–3.43 (m, 4H, overlapped resonances of H4, 6 and 3), 3.26 (s, 3H, $2OCH_3$), 3.20 (s,

3H, 6OCH_3), 3.19 (s, 3H, 1OCH_3), 3.17 (m, 1H, H5), 3.05 (2d, 1H, $J_{2,1}$ = 3.5 Hz, H2).

Anal. calcd for $C_9H_{18}O_6$: C, 48.48; H, 8.10. Found: C, 48.38; H, 7.98.

Methyl 3,6-di-*O*-methyl-α-D-glucopyranoside, 1c

Methyl 2,4-di-*O*-benzyl-3-*O*-methyl-α-D-glucopyranoside (Kovac, 1973) (5.0 g) was dissolved in 60 mL of dry DMSO; to this solution 0.61 g of NaH was added. After stirring for 1 h at room temperature, 3 mL of CH_3I was added dropwise over a period of 30 min. Stirring was continued overnight, and then excess NaH was decomposed with 3 mL of CH_3OH. The mixture was diluted with 300 mL of H_2O and extracted with 3 × 100 mL of ether. The combined ether extracts were washed with H_2O, dried ($MgSO_4$), and the solvent was evaporated. The resulting colorless syrup was chromatographed on silica gel (hexane/ethyl acetate, 1:1, R_f = 0.5) to give 4.85 g of a syrup (93.6%), $[\alpha]_{20}$ 52.6° (c, 0.698, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ7.28–7.36 (m, 10H, aromatic protons), 4.86, 4.78, 4.63, 4.59 (all d, 4 H, benzylic protons), 4.55 (d, 1H, $J_{1,2}$ = 3.5 Hz, H1), 3.68 (s, 3H, 3OCH_3), 3.64–3.67 (m, 2H, overlapped resonances of H5 and H3), 3.56 (2d, 1H, H6a), 3.47–3.50 (m, 2H, overlapped resonances of H4 and H6b), 3.42 (2d, 1H, $J_{2,1}$ = 3.5 Hz, H2), 3.33 (s, 3H, 6OCH_3), 3.31 (s, 3H, 1OCH_3); ^{13}C NMR δ98.34 (C1), 83.85 (C3), 79.51 (C2), 77.50 (C4), 74.96 and 73.35 (benzylic carbons), 70.91 (C6), 69.77 (C5), 61.22 (C1 OCH_3), 59.17 (C6 OCH_3), 55.15 (C1 OCH_3); resonances of aromatic carbons are excluded.

Anal. calcd for $C_{23}H_{30}O_6$: C, 68.65; H, 7.46. Found: C, 68.37; H, 7.52.

The catalytic transfer hydrogenation of the above compound was performed as reported by Rao & Perlin (1980): 0.5 g of the compound was dissolved in 50 mL of CH_3OH containing 10% of formic acid. This solution was added to a stirred suspension of 2g of 10% Pd–C in 50 mL of the same solvent mixture. The reaction (monitored by TLC) was maintained under a N_2 atmosphere and was completed in 3 h. The catalyst was filtered and washed with CH_3OH. The filtrates were combined and evaporated. The residue was purified by column chromatography ($CHCl_3$: CH_3OH, 10:1, R_f, 0.3) to give 0.23 g of a syrup (85%); $[\alpha]_{20}$ 116.8° (c, 0.754, H_2O); ^{1}H NMR (D_2O) δ4.56 (d, 1H, $J_{1,2}$ = 3.70 Hz, H1), 3.55 (m, 1H, H5), 3.39–3.49 (m, 3H, overlapped resonances of H6a, 6b and 2), 3.38 (s, 3H, 3OCH_3), 3.30–3.27 (m, 2H, overlapped resonances of H4 and H3), 3.20 (s, 3H, 6OCH_3), 3.18 (s, 3H, 1OCH_3).

Anal. calcd for $C_9H_{18}O_6$: C, 48.64; H, 8.10. Found: C, 48.90; H, 8.13.

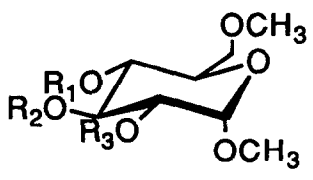
Methyl 4,6-di-*O*-methyl-α-D-glucopyranoside, 1d

It was prepared by the method of Bell and Lorber (1940). The crude product was purified by column chromatography instead of by vacuum distillation. It was eluted with $CHCl_3$ and CH_3OH (11:1); fractions having R_f = 0.2 were combined and concentrated. $[\alpha]_{20}$ 156.3° (c,1.077 g, $CHCl_3$), lit (Kovac, 1973) $[\alpha]_{20}$ 156.8°; 1H NMR (D_2O) δ4.55 (d,1H, $J_{1,2}$ = 3.40 Hz, H1), 3.45–3.52 (m, 4H, overlapped resonances of H6, H5, H3), 3.34 (2d, 1H, $J_{2,1}$ = 3.40 Hz, H2), 3.32 (s, 3H, 4-OCH_3), 3.19 (s,3H, 6OCH_3), 3.17 (s, 3H, 1OCH_3), 2.96 (t, 1H, $J_{3,4}$ = 8.3 Hz, H-4).

Anal. calcd for $C_9H_{18}O_6$: C, 48.64; H, 8.10. Found: C, 48.64; H, 8.20.

Methyl 2,3,6-tri-*O*-methyl-α-D-glucopyranoside, 1e

It was prepared by the procedure described by Jackson *et al.* (1982).



	R_1	R_2	R_3
1a	H	H	H
b	H	H	CH_3
c	H	CH_3	H
d	CH_3	H	H
e	H	CH_3	CH_3

Scheme 1

RESULTS AND DISCUSSION

Norrman (1968) reported using dimethyl sulfate in concentrated NaOH solution for the partial methylation, to DS of 1.35, of dextran from *L. mesenteroides* B-512. Structural analysis was done using gas chromatography, after complete hydrolysis, and trimethylsilylation of the methylated product. Since we desired a product with a DS range of up to 1 we adapted the approach of Kobayashi and Sumimoto (1981) who employed Hakomori's methylsulfinylmethyl anion in DMSO for deprotonation of dextran. We prepared the anion using NaH as the limiting reagent. Iodomethane was the methylating agent.

For our structural analysis of the methylation product, which employed ^{13}C NMR spectroscopy, the signal assignments made for dextran by Kobayashi and Sumimoto (1981) were confirmed. This was accomplished by 1-D and 2-D analyses of the ^{1}H and ^{13}C spectra of a dextran sample having an average molecular weight of 1500. The ^{13}C signal assignments agreed with those made earlier (Kobayashi & Sumimoto, 1981), and with those published while this work was underway (McIntyre & Vogel, 1991; Shimamura *et al.*, 1991). In addition, methyl 6-*O*-methyl-α-D-glucopyranoside, **1a**, was employed as a model for dextran, and monomethyl and dimethyl derivatives **1b-e** were prepared for determination of 'Δδ' values, the change in chemical shift of ring carbons imparted by *O*-methyl substitution. The ^{13}C chemical shifts of

compounds **1**, along with the $\Delta\delta$ values, are collected in Table 1.

The $\Delta\delta$ values of Table 1 were used to assign ^{13}C NMR signals of monomethylated and dimethylated residues in dextran samples. These are collected in Table 2. For signal assignments to the 2,4- and 3,4-dimethylated residues, the $\Delta\delta$ values of the appropriate monomethylated glucosides were summed. (Summing the $\Delta\delta$ values of **1b** and **1c** satisfactorily predicts the chemical shifts of compound **1e**, for example.) Our integration of the $COCH_3$ signals, using the anomeric carbon signals as the internal reference, gave the following product ratios for the dextran sample with DS = 0.72: 2-OCH_3 : 4-OCH_3 : 3-OCH_3 : 2,4 di-OCH_3 : 2,3 di-OCH_3 : 3,4 di-OCH_3 = 4.3: 1.7: 1.0: 1.0: ~0.7: ~0.7; for a sample with DS = 0.17: 2-OCH_3 : 4-OCH_3 : 3-OCH_3 : 4.1: 1.5: 1.0.

The rate of agglutination of suspensions of *S. cricetus* and *S. sobrinus* by high molecular weight dextran has proved to be a useful assay of glycan recognition by the glucan-binding lectin (GBL) of the bacteria (Drake *et al.*, 1988). This assay has also been used to demonstrate that dextran of MW of 10^4 inhibits this aggregation, a result which indicates recognition also of this dextran by the lectin. Interestingly, an α-(1→6) glucose octamer is the minimum oligomer size required for significant inhibitory activity. That is, methyl α-D-glucopyranoside and other monosaccharides are not recognized by GBL (Drake *et al.*, 1988; Landale *et al.*, 1987; Baumann *et al.*, 1993). The respective methylated dextrans exhibit significantly reduced effectiveness of agglutination, and inhibition of agglutination (Wang *et al.*, 1995). For example, 2×10^6 MW dextran having a DS of 0.17 exhibited a 28-fold decrease in rate of bacterial agglutination. Effectively, this indicates loss of recognition by the lectin. With a DS of 0.17 a 2-OCH_3, the major product, would be found every 9 residues, on average; a 2-OCH_3 or a 4-OCH_3, the two major products, would be found every 6.4 residues. Given the large binding site, the effect on biological activity by this low degree of methylation is striking. That this change is due to loss of only one hydrogen-bonding locus per binding site seems unlikely.

Statistical mechanical and MO studies of the conformation of dextran (Brant & Burton, 1981; Middlemiss *et al.*, 1985; Tvaroska *et al.*, 1978; Bock & Pedersen, 1988) predict no long range order beyond 'a tendency toward pseudohelical order' (Brant & Burton, 1981). Detailed semiempirical MO (Tvaroska *et al.*, 1990) and molecular mechanics calculations on the disaccharide isomaltose predict that the gg conformation about angle ω(O5-C5-C6-O6) is the most abundant, and this is supported by measurements of the appropriate $^3J_{H,H}$ (Middlemiss *et al.*, 1985) and $^3J_{C,H}$ (Bock & Pedersen,

Table 1. ^{13}C Chemical shift assignments and $\Delta\delta$ values[a] for O–methylated methyl α-D-glucopyranosides 1

Compound	C1	C2	C3	C4	C5	C6	1-OCH_3	6-OCH_3	*n*-OCH_3
1a	101.81	73.61	75.43	72.58	72.09	73.41	57.63	60.91	—
1b	98.98 (−2.83)	82.66 (9.05)	74.65 (−0.78)	72.36 (−0.22)	72.10 (0.01)	73.37 (−0.04)	57.35	60.98	60.32
1c	102.03 (0.22)	73.35 (−0.26)	85.57 (10.14)	72.88 (0.30)	71.78 (−0.31)	73.52 (0.11)	57.88	61.20	62.70
1d	101.76 (−0.05)	73.75 (0.14)	75.26 (−0.17)	81.93 (9.35)	71.70 (−0.39)	73.26 (−0.15)	57.72	60.95	62.58
1e	99.28 (−2.53)	82.41 (8.80)	84.77 (9.34)	71.74 (−0.84)	72.68 (0.59)	73.41 (0.00)	57.60	61.17	b

[a]δ 1b–e–δ 1a; in parentheses. [b]2-OCH_3: 60.34; 3-OCH_3: 62.64.

Table 2. ^{13}C Chemical shift assignments of glucose residues in dextran and partially methylated dextrans

Glucose Residue in Dextran	Carbon Chemical Shifts[a]					
	C1	C2	C3	C4	C5	C6
Unmethylated	100.16	73.86	75.85	71.97	71.64	67.97
2-OCH_3-[b]	97.57	82.82	74.93	—	—	—
3-OCH_3-[c]	100.74	73.46	85.83	—	—	68.48
4-OCH_3-[d]	100.06[e]	—	75.33	81.72	—	—
2,3-di-OCH_3-	98.15	82.51[f]	85.00	—	—	—
2,4-di-OCH_3-	100.06[e]	83.21[e]	74.46	81.25	—	—
3,4-di-OCH_3-	—	—	85.66	82.51[f]	—	—

[a]Referenced to DSS; solvent D_2O; average of at least four spectra; [b]OCH_3; δ60.52; [c]OCH_3: δ62.82; [d]OCH_3: δ62.34; [e]shoulder on nearest reported signal; [f]tentative assignment.

1988) values of isomaltose. In contrast, our preliminary MM2 calculations on α-(1→6) linked glucose triads and tetrads, as models for dextran, predict that turns in the chain imparted by the angle ω sequence of gt→gg (nonreducing→reducing) are more stable than the extended conformation having gg→gt, or sequential gg conformations. Presumably, this results from attractive forces (van der Waals, hydrogen bonding) operating between non-adjacent glucose residues. A stereo view of such a turn is shown in Fig. 1. Electron and X-ray diffraction studies of two polymorphs of dextran have shown that these possess sequential gt conformations about angle ω, and have 02-04 H-bonds between residues spaced 1,3 (Guizard *et al.*, 1984, 1985). Also, the linkage angle ω of crystalline panose, α-Glc(1→6)α-Glc(1→4)Glc, has the gt conformation with the turn stabilized by a hydrogen bond between the nonadjacent reducing and nonreducing terminal residues (Imberty & Pérez, 1988; Jeffrey & Bin, 1991). Moreover, this conformation, with the same stabilizing features, is maintained in aqueous solution (Sheng *et al.*, 1994; Poppe *et al.*, 1994). Thus, structural data on dextran, and on a trisaccharide model support the prediction of our preliminary MM2 calculations (see also Baumann *et al.*, 1993). Our calculations also indicate that monomethylation at O2 or O4 would destabilize the gtgg conformation the most. The implication is that methylation at O2 and O4 destabilizes a conformational feature of dextran that is responsible for recognition by the lectin.

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