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Carbohydrate Research 266 (1995) 229–235

CARBOHYDRATE
RESEARCH

NMR studies of dextran oligomer interactions with model polyphenols ☆

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Received 2 June 1994; accepted 22 August 1994

Abstract

The interaction of bacterial dextrans with polyphenols has been implicated in the formation of dental plaque. Therefore, the NMR spectra of the (1 → 6)-α-linked dextran oligomers, (glucose)$_n$ ($n = 1, 2, 3, > 3$), have been assigned using two-dimensional methods. Addition of a model polyphenol, propyl gallate, causes only very small changes in the NMR chemical shifts of isomaltose ($n = 2$) and polymers of average length up to 8.2, indicating little apparent binding under these conditions. Titration of dextrans with a model nonhydrolysable proanthocyanidin tannin, (−)-epicatechin, produced no significant chemical shift changes. These results suggest that the function of bacterial dextrans in modulating the polyphenol content of plaque is at most indirect.

Keywords: Dextran; NMR; Tannin; Polyphenol; Binding

1. Introduction

Dental caries is caused by the adhesion of pathogenic bacteria to teeth and gums and subsequent attack on the gums by the bacteria. It has been demonstrated that the pathogenic bacteria are unusual among the oral bacteria in that they produce large amounts of extracellular dextrans [(1 → 6)-α-linked glucose polymers], and that these dextrans are instrumental in the adhesion of the bacteria to tooth enamel [1]. Large amounts of dextran are found in dental plaque [1]. The other major constituents of plaque are calcium, phosphate, the salivary proline-rich proteins, and polyphenols (plant tannins), which form a large part of the dry weight of many common foodstuffs including sorghum, millet, unripe fruits,

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☆ Abbreviations: TSP, (2,2,3,3-d_4)-trimethylsilylpropionate; COSY, correlated 2D spectroscopy; TOCSY, total correlated 2D spectroscopy; PRP, proline-rich protein.

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SSDI 0008-6215(94)00273-8

wine, beer, tea, and coffee [2]. It is thought that the dextrans and salivary proteins can interact to form a matrix, which acts as the core of dental plaque, and that the plaque mass is then increased by accumulation of the other constituents. It has been shown that salivary proline-rich proteins can bind to tooth enamel, and that they can bind tannins strongly enough to produce high molecular weight insoluble complexes [3,4]. However, the role of the dextrans in this process is not clear [5]; in particular, no direct role of the dextrans in polyphenol binding has been shown, apart from the formation of inclusion complexes of cyclodextrin with polyphenols [6]. We have therefore studied the interaction of dextrans with the model polyphenols, propyl gallate, and (−)-epicatechin, which can be considered as the core constituents of the hydrolysable and nonhydrolysable tannins, respectively.

2. Experimental

Materials.—Glucose, isomaltose (6-*O*-α-D-glucopyranosyl-D-glucose), isomaltotriose [α-D-glucosyl-(1→6)-α-D-glucosyl-(1→6)-D-glucose], dextran (from *Leuconostoc mesenteroides*, average molecular weight 9300), dextranase (from *Penicillium* spp.), deuterium oxide, and perdeuterated dimethylsulfoxide were purchased from Sigma. Biogel P2 was purchased from Bio-Rad. Propyl gallate and (−)-epicatechin were gifts from Professor E. Haslam, Department of Chemistry, University of Sheffield.

Preparation of low-molecular weight dextrans.—Dextran (120 μmol, 1.2 g) was digested with 2 units of dextranase for 1–2 h at room temperature. The products were immediately purified by passage through a Biogel P2 column (1.6×65 cm). The sugars did not absorb significantly at 254 or 280 nm, and so they were detected using the method of Rick and Segbauer [7], which is based on the reaction of reducing sugars with 3,5-dinitrosalicylic acid in potassium tartrate. The length of the dextran polymer was measured by ^{1}H NMR spectroscopy, comparing the integral of the nonreducing anomeric signal to the reducing anomeric signals. The degree of polymerisation quoted is therefore a mean value, but from analysis of different fractions we expect the spread of chain lengths to be almost entirely the value quoted ± 1.

NMR experiments.—Experiments were carried out on a Bruker AMX-500 spectrometer in 9:1 D_2O–deuterated dimethyl sulfoxide (M_2SO), using trimethylsilylpropionate (TSP) as a reference. Most experiments used TSP as an internal reference, but crucial experiments with tannin titrations had TSP as an external reference in a capillary tube, to remove effects of the tannin on the TSP reference shift. The binding studies used 1 mM solutions of sugars, with the addition of small amounts of 50 mM solutions of tannin. Studies were carried out at several temperatures in the range 283–300 K.

Two-dimensional spectra were carried out at 300 K using approximately 5 mM solutions. Correlated spectra were acquired using a double-quantum filtered COSY, and TOCSY spectra used a DIPSI-2 spin lock of 11.8 kHz power, 150 ms duration, with a Hahn echo before the acquisition, to facilitate time-domain filtering of the data, reduce the residual water signal, and produce a flatter baseplane [8]. Two-dimensional spectra were acquired into 4096 complex points over a spectral width of 6250 Hz in both dimensions. Quadrature detection in the indirect dimension (F_1) was achieved using time-proportional phase incrementation using 512 real increments. Spectra were transformed into 2048×2048 points by

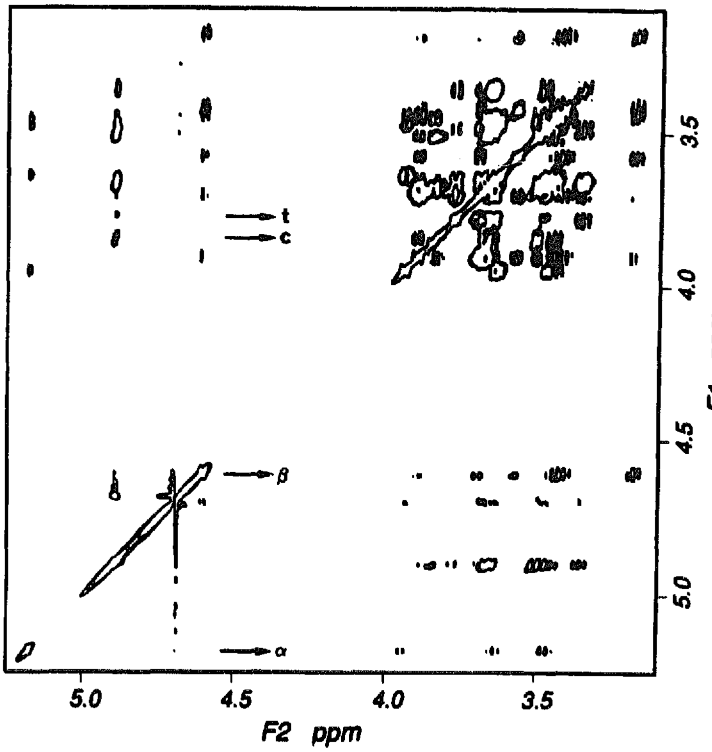

Fig. 1. TOCSY spectrum of 5 mM isomaltotriose in 90% D_2O–10% Me_2SO-d_6, 300 K. The arrows mark the positions where cross-sections were taken for Fig. 2.

removing 1024 points from each side of the F_2 spectrum (i.e., the time domain data were acquired using oversampling [9]), and zero filling three times in F_1. Sinebell windows were used. All spectra were processed using FELIX (BIOSYM) and viewed on Silicon Graphics workstations.

3. Results

Preparation of oligodextrans.—Oligodextrans were prepared by limited hydrolysis of commercial dextran and purified by two applications to a size exclusion column.

Assignment of the spectra.—Spectra were assigned largely from COSY and TOCSY spectra. Despite the complexity and high degree of overlap of the spectra, subspectra from the different contributing saccharide units were obtained by use of suitable TOCSY cross-sections from H-1′ or occasionally from H-6′, H-6″, or H-5′. As an example, Fig. 1 shows the complete TOCSY spectrum of isomaltotriose, and Fig. 2 shows cross-sections through the TOCSY spectrum, each containing contributions from just one monomer unit. Within each monosaccharide unit, assignments were made from COSY spectra, and by consideration of coupling constants and strong coupling patterns. The assignments were made independently for each oligosaccharide, and confirmed by comparisons between different saccharides.

$(1\rightarrow 6)$-α-Linked dextrans have a good deal of conformational flexibility, which may explain why in all cases the nonreducing sugars from molecules with α- and β-anomeric

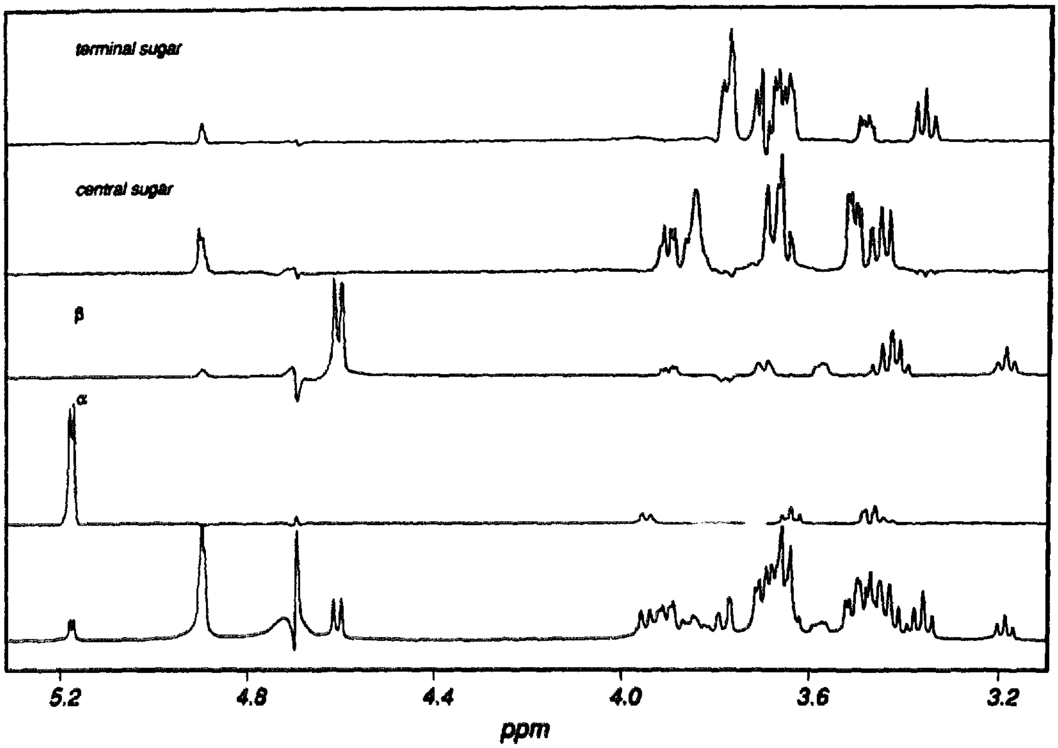

Fig. 2. Cross-sections through the TOCSY spectrum of Fig. 1, showing the individual sugar residues. The bottom trace shows the complete spectrum.

configurations at the reducing end gave totally coincident sets of signals. The complete assignments for glucose, isomaltose, and isomaltotriose are presented in Table 1. In longer dextrans, the signals from nonterminal sugars all coincided with the signals of the central sugar in isomaltotriose.

Table 1
Chemical shift assignment for (1 → 6)-α-linked dextrans

Residue	H-1'	H-2'	H-3'	H-4'	H-5'	H-6'	H-6"
Glucose							
α anomer	5.24	3.54	3.72	3.41	3.84	3.85	3.73
β anomer	4.65	3.25	3.49	3.39	3.47	3.90	3.73
Isomaltose							
α anomer	5.23	3.53	3.72	3.49	3.70	4.00	3.52
β anomer	4.66	3.24	3.47	3.52	3.63	3.76	3.96
Nonreducing sugar	4.95	3.54	3.73	3.42	3.73	3.85	3.73
Isomaltotriose							
α anomer	5.23	3.53	3.69	3.49	3.69	4.00	3.50
β anomer	4.66	3.24	3.46	3.49	3.63	3.75	3.95
Central sugar [a]	4.95	3.56	3.72	3.50	3.73	3.96	3.89
Terminal sugar	4.95	3.54	3.71	3.41	3.73	3.83	3.76

[a] These chemical shifts apply to all central sugars in longer dextrans.

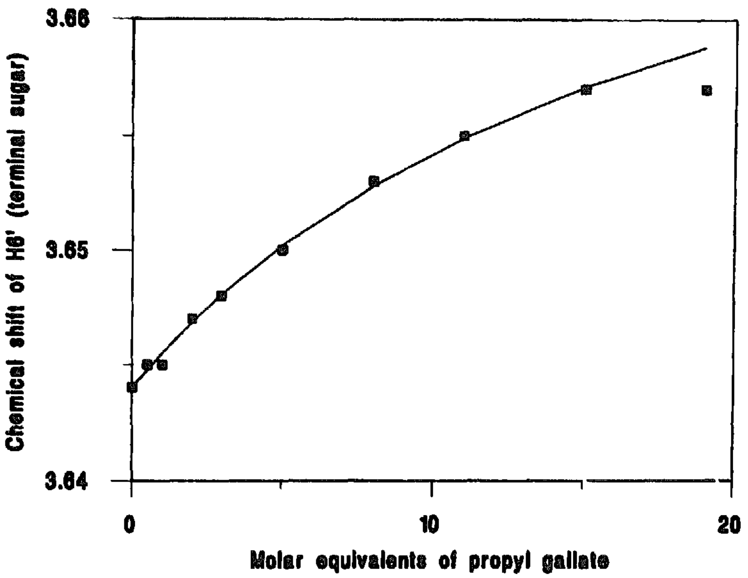

Fig. 3. Chemical shifts of the H-6′ resonance of the terminal sugar of isomaltotriose with addition of propyl gallate. The squares mark the experimental points, and the curve is the best-fit curve, corresponding to a dissociation constant of 29 mM and a maximum shift change at saturation of 0.046 ppm.

The longer dextrans prepared by hydrolysis of commercial dextran contained an extra anomeric proton signal at ca. 5.32 ppm. This signal probably derives from (1 → 3)-α-linked glucoses [10,11].

Titrations with propyl gallate.—In preliminary experiments, it was determined that precipitation occurred on adding propyl gallate or epicatechin at high concentration to dextrans. Both tannins have a relatively low solubility in water. For these reasons, it was decided to carry out the titration experiments in 10% perdeuterated Me_2SO in D_2O. This solvent system is the same as that previously used by us to study peptide–tannin interactions; in these studies we have shown that the addition of 10% Me_2SO to water has little effect on peptide–tannin interactions [12,13]. Other workers have shown that Me_2SO–water mixtures at low temperature (as used here) have similar properties (for example, viscosity and dielectric constant) to those of water at physiological temperatures [14].

Titrations were carried out using dextran polymers, with a degree of polymerisation (as measured by NMR) ranging from 2 up to 8.2. The results were similar for all polymers measured. Addition of propyl gallate up to 20 equivalents caused shifts (relative to external TSP) of no more than 0.02 ppm in sugar resonances (Fig. 3). The shifts observed were similar for all resolved sugar resonances, and in most cases could be fitted to a simple 1:1 saturation curve, as illustrated in Fig. 3 [12]. The 12 protons that could be fitted gave apparent dissociation constants of 41 ± 16 mM. Using this fitting method, the apparent chemical shift change on 100% saturation was ca. 0.05 ppm for all protons fitted (Table 2).

Titrations with epicatechin.—Titrations were carried out using epicatechin with a range of different length dextrans, using up to 50 equivalents of epicatechin. The largest chemical shift change was no greater than 0.01 ppm; for example, with isomaltotriose the observed chemical shift change after 30 equivalents was 0.004 ± 0.0016 ppm measured over 19 resolved resonances, while with an 8.2mer the observed shift change was 0.009 ± 0.003 ppm. There was no observable distinction between the shifts seen for protons from different

Table 2
Typical chemical shift changes and calculated binding constants for the (glucose)$_{8.2}$:propyl gallate titration

Proton	Shift change after 19 equivalents propyl gallate	Values obtained from fitting	
		$\Delta\delta^{a}_{max}$ (ppm)	K_d (mM)
H-1′ (1→3 link)	0.011	0.039	30
H-1′ (c and t)	0.007	0.040	60
H-6′ (c)	0.006	0.040	61
H-6″ (c)	0.012	0.061	47
H-6′ (t)	0.013	0.046	28
H-2′ (c)	0.011	0.073	61
H-3′ (β)	0.015	0.049	26
H-4′ (t)	0.013	0.037	21
H-2′ (β)	0.018	0.061	28

[a] Maximum shift change on 100% saturation.

saccharide units. Small changes in chemical shift of the tannin were seen both with epicatechin and with propyl gallate, but these chemical shift changes could be ascribed to self-association phenomena [12].

4. Discussion

There are two classes of polyphenols: the hydrolysable tannins, which are gallic acid esters of glucose, often oxidatively cross-linked; and the nonhydrolysable tannins or proanthocyanidins, which are polymers of epicatechin, with additional galloyl ester groups [2]. The two tannins used in this study are therefore representative of these two classes. More complex hydrolysable tannins could not readily be used because the NMR signals from the glucose moiety of the tannin overlap with the saccharide signals of the dextran. We have previously studied the interactions of salivary peptides with model hydrolysable tannins and with proanthocyanidins, and have shown that NMR chemical shift changes can be used to follow the binding, and reliably to calculate dissociation constants at specific sites [11].

The maximum chemical shift change expected on complexation of an aromatic ring with a ligand is up to 2 ppm. The chemical shift changes seen here are orders of magnitude smaller, even when extrapolated to 100% saturation of binding. This suggests that there is no appreciable binding occurring. The uniformity of the chemical shift changes seen further supports this suggestion. However, the chemical shift changes observed are real (because an external standard was used to measure the shifts), different for each oligosaccharide, and saturable, as illustrated in Fig. 3. There is a tendency for the observed changes to be larger for the longer dextrans. We therefore tentatively ascribe the chemical shift changes largely to conformational changes in the saccharides (e.g., a tendency to curl up in the more hydrophobic and ordered tannin-rich solutions) rather than a specific binding event.

There is no evidence that longer dextran polymers bind any more tightly to tannins [15]. The only polysaccharides for which significant tannin binding is well documented are those containing cavities, such as cyclodextrins, or those that readily form such cavities, such as amylose, which readily forms a helical structure. By contrast, dextrans are typically unstructured. We therefore believe that these results also hold true for the longer polymers typically found in the mouth.

The oral cavity contains a complex mixture of species, and in particular high concentrations of salivary proline-rich peptides (PRPs), which make up ca. 70% of total salivary protein, and dextrans produced by oral bacteria. The tooth surface is coated with PRPs, which help to maintain calcium homeostasis. It is known that dental plaque consists largely of PRPs, inorganic ions (particularly calcium and phosphate), dextrans, and tannins. The amount of plaque formed depends on the concentration of dextran in the mouth. The results presented here suggest that there is no direct interaction between dextrans and tannin. The role of dextrans is therefore more likely to be in binding to PRPs, and thereby modulating the PRP–tannin interactions. Further work is in progress to investigate this possibility.

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