Synthesis of Xylo- and Mannodextran

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Synthetic antigens are of importance in immunochemistry for studying cross reactions and to obtain antibodies with a high specificity. Such antigens may be prepared by attaching sugar residues to proteins by coupling the corresponding diazotised *p*-aminophenyl glycosides [1] or by conjugating oligosaccharide aldonic acids by the mixed anhydride reaction.[2] Such modified antigens differ considerably from natural polysaccharide antigens. The introduction of a protein backbone and the presence of unnatural coupling groups may cause immunological reactions which are not typical for natural polysaccharide antigens. Therefore it would be preferable to prepare artificial polysaccharide antigens having structures the least different from those of natural antigens. We have initiated such studies and in the present paper we describe a method by which single sugar residues may be glycosidically linked to polysaccharides.

Some years ago we reported [3] the transfer of β-D-xylopyranose residues from phenyl β-D-xylopyranoside to different alcohols such as methanol, glycerol, amylose, and cellulose by alkaline transglycosidation. The requirements for this reaction are, first, a *trans* relationsship between the phenoxyl group and the hydroxyl at C-2 in the glycoside, allowing the formation of the reactive 1,2-anhydro sugar, and second, absence of intramolecular reaction in this anhydro sugar. Glycosides of low molecular weight alcohols are prepared in high yields by using the alcohol as solvent. The reaction with polysaccharides must be performed in concentrated aqueous solution and a considerable part of the 1,2-anhydride intermediate is lost through side reactions with water and with the hydroxyl groups of the starting material or its reaction products.

The immunochemical properties of dextrans have been extensively studied [1] and it was therefore natural to choose this polysaccharide as the backbone, to which new sugar residues were to be attached. Syntheses were performed with phenyl β-D-xylopyranoside and *p*-nitrophenyl α-D-mannopyranoside. It was first demonstrated that the latter compound gave a good yield of methyl α-D-mannopyranoside when treated with sodium methoxide in methanol.

Xylodextran containing 10 % D-xylose residues and mannodextran with 5 % D-mannose residues were prepared from a concentrated solution of dextran and the phenyl glycoside in aqueous sodium hydroxide. Methylation analyses of the substituted dextrans and the original dextran showed that the β-D-xylopyranose and α-D-mannopyranose residues have been introduced as endgroups mainly in the 2-positions of the dextran (Table 1). These results therefore show, as expected, that the reaction occurs with the most acidic hydroxyl groups. Quantitative precipitin reactions between concanavalin A and mannodextran and between concanavalin A and the original dextran showed, as expected, that the mannodextran had a better precipitating capacity than the original dextran (Fig. 1).[4]

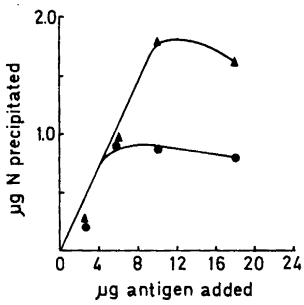

Fig. 1. Precipitation of concanavalin A (8.2 µg N) with dextran NRRL B−512 (●) and mannodextran (▲) in phosphate buffered saline (pH 7.2); total volume 100 µl.

The experiments demonstrate the possibility of attaching sugar residues to dextran by alkaline transglycosylation. The hydroxyl groups in the attached residues may also react with the 1,2-anhydro sugar, yielding di- and oligosaccharide residues. Due to the low degree of substitution this side reaction was insignificant but may become more serious at higher degrees of

Table 1.

Sugar[a]	Molar percentage			Relative retention time[b]
	Dextran	Xylodextran	Mannodextran	
2,3,4-Tri-*O*-Me-Xyl	—	8.2	—	0.68
2,3,4,6-Tetra-*O*-Me-Man	—	—	4.2	1.00[c]
2,3,4,6-Tetra-*O*-Me-G	4.2	3.7	3.8	1.00[c]
2,3,4-Tri-*O*-Me-G	91.5	76.1	83.9	2.50
2,4-Di-*O*-Me-G	4.3	3.7	3.9	5.10
3,4-Di-*O*-Me-G	—	8.3	4.2	5.27

[a] G = D-glucose, Xyl = D-xylose, Man = D-mannose, Me = methyl.
[b] As alditol acetate on column (a) relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.
[c] 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol and 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-mannitol were separated on column (h) with *T*-values 1.00 and 1.08, respectively.

substitution or if higher oligosaccharides were transferred in similar reactions. One limitation of the method is that only one anomeric form of the sugar residue, that having a *trans* relationship between the glycosidic oxygen atom and the hydroxyl at C-2, may be attached. The severe loss of 1,2-anhydro sugar in side reactions is also a limitation especially if the transglycosylation involves a less readily available sugar.

Experimental. GLC was performed in a Perkin-Elmer model 900 instrument, using the following columns: (a) 3 % nitrile silicone-polyester copolymer (ECNSS—M) at 200° for alditol acetates and at 165° for partially methylated alditol acetates (Scot column); (b) polyphenyl ether (OS 138) at 200° for partially methylated alditol acetates. For GLC—MS, the mixtures of alditol acetates, dissolved in acetone, were injected into a Perkin-Elmer 270 gas chromatograph-mass spectrometer. The mass spectra were recorded at a manifold temperature of 200°, ionisation potential of 60 eV, ionisation current 80 μA and an ion source temperature of 80°.

Methylation analysis. The polysaccharide was fully methylated by the method of Hakomori.[5] The methylated polysaccharide (5 mg) was treated for 2 h with 90 % formic acid at 100°, and then hydrolysed with 0.25 M sulphuric acid at 100° for 18 h. The hydrolysed sugars were converted into alditol acetates and analysed by GLC [6] on columns (a) and (b). The components were identified by GLC—MS.[7] Table 1).

Preparation of substituted dextrans. Dextran NRRL B—512 (0.5 g) and phenyl glycoside (phenyl β-D-xylopyranoside, *p*-nitrophenyl α-D-mannopyranoside) (1 g) were mixed together with enough 6 M aqueous sodium hydroxide (about 0.5 ml) to form a thick syrup and heated for 36 h at 100° under nitrogen. The resulting dark syrup was dissolved in water, neutralised with dilute acetic acid and dialysed. The substituted dextran was then precipitated by addition of ethanol.

Part of the material (2 mg) was hydrolysed with 0.25 M sulphuric acid and neutralised with barium carbonate. The sugars in the hydrolysate were converted into alditol acetates and analysed by GLC on column (a).[8] The identities of the sugars were confirmed by mass spectrometry.[9] *Quantitative precipitin analyses* [1] were carried out by a microprecipitin technique using the ninhydrin procedure for nitrogen determination.[10]

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Polysaccharides Elaborated by *Fomes annosus* (Fr.) Cooke

I. A Water-soluble Acidic Polysaccharide from the Fruit Bodies

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In a recent publication,[1] studies on the structure of two acidic polysaccharides isolated from *Polyporus fomentarius* and *Polyporus igniarius*, were reported. In the present communication, studies are reported on a similar polysaccharide isolated from *Fomes annosus (Polyporus annosus)*, another species which also causes severe wood destruction.

Fruit bodies of *F. annosus* were harvested locally. The isolation of water-soluble polysaccharides and their separation into neutral and acidic polysaccharides was performed as previously described.[2] Studies on the neutral polysaccharides will be reported separately. The acidic material was further purified by column chromatography on DEAE-Sephadex in the acetate form.[1] The purified acidic polysaccharide, $[\alpha]_{578}^{20} - 37°$ (*c* 0.13, H_2O), yielded on hydrolysis D-glucose and D-glucuronic acid. This glucuronoglucan contained *ca.* 35 % uronic acid residues, as determined by the carbazole method,[3] with correction for the colour reaction given by the glucose residues. The following method was also applied for determining glucuronic acid content. The glucuronoglucan was partially hydrolysed and the mixture of mono- and acidic oligosaccharides was treated with methanolic hydrogen chloride and then converted to ether-soluble trimethylsilyl-derivatives. Finally, the esterified glucuronic acid residues were reduced with lithium aluminiumdeuteride.[4] By this procedure, the D-glucuronic acid residues were transformed into D-glucose residues deuterated at C-6 ($-CD_2OH$). The mixture of deuterated and unlabelled glucose, obtained on subsequent hydrolysis, was reduced with sodium borohydride, acetylated and analysed by GLC[5]—mass spectrometry.[6] Fission of unlabelled D-glucitol hexaacetate between C-3 and C-4, yields the primary fragment, *m/e* 217, from both halves of the molecule; whereas, the deuterated compound, by the same fission should give equal amounts of an *m/e* 217 fragment from C-1 to C-3 and an *m/e* 219 fragment from C-4 to C-6. From the relative intensities of the peaks with *m/e* 219 and *m/e* 217, the molar percentage of deuterated glucitol acetate (*i.e.* glucuronic acid) was estimated as being approximately 30 %.

The polysaccharide was methylated as previously described.[1] Part of the methylated polysaccharide was hydrolysed, the

Table 1. Methyl ethers obtained from the hydrolysate of A: methylated glucuronoglucan, B: methylated-reduced glucuronoglucan.

Sugars	T^a	mole %	
		A	B
2,3,4,6-Tetra-*O*-Me-D-G	1.00	17	12
2,4,6-Tri-*O*-Me-D-G	1.95	18	17
2,3,4-Tri-*O*-Me-D-G	2.48	41	31 (6)[b]
2,4-Di-*O*-Me-D-G	5.10	24	17
2,3-Di-*O*-Me-D-G	5.40	—	23

[a] Retention times of the corresponding alditol acetate on the ECNSS-M column relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.

[b] The figure in the bracket represents the 2,3,4-tri-O-methyl-D-glucose, derived from D-glucuronic acid residues.