Oxidation of Dextran NRRL B-512 with Sodium Nitrite in Phosphoric Acid

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Primary alcohol functions in polysaccharides can be oxidized with nitrous acid or nitrogen dioxide to carboxylic acids. The nitrogen dioxide oxidation of cellulose under various conditions has been extensively investigated.[1–5] In most cases, some depolymerisation takes place. In an improved oxidation procedure developed by T. Painter,[1] the polysaccharide is oxidized in the foam that is formed when sodium nitrite is added to the viscous solution of the polysaccharide in orthophosphoric acid. The method has been used to oxidize primary alcohol groups in different polysaccharides for the synthesis of heparinoids.[6] In addition to the oxidation of the primary alcohol groups, some evidence indicating oxidation of secondary alcohol groups, resulting in small amounts of glycosulose residues has been reported.[1,4,7]

Dextran elaborated by *Leuconostoc mesenteroides* NRRL B-512 is a (1→6)-linked α-D-glucan with side chains (~5 %) linked to C-3 of the D-glucose residues.[8] When dextran was treated with aqueous bromine,[9] C-2 or C-4 in the glucose residues was oxidized, resulting in 2- and 4-glycosulose residues. Oxidation with dimethyl sulfoxide–acetic anhydride occured mainly at C-3 in the glucose residues.[10]

The purpose of this investigation was to study the oxidation of secondary alcohol groups in polysaccharides with sodium nitrite in orthophosphoric acid. Dextran was chosen, since it is readily available and has few primary hydroxyl groups.

Dextran was oxidized with sodium nitrite (4.7 or 7.0 mol/mol glucose residue) in phosphoric acid, yielding polymers containing carboxylic acid and carbonyl groups (oxidized dextrans I and II, respectively). Gel filtration of the reduced[11] – oxidized dextrans I and II on Superose 6B established that little depolymerisation occurred during the oxidations or the work-up procedure.

The oxidized dextrans contained ~5 % uronic acid (Table 1) residues, as determined by decarboxylation,[12] indicating quantitative oxidation of the primary alcohol groups of the non-reducing terminal residues (~5 %).

A portion of each oxidized polymer was treated with methoxylamine hydrochloride to convert the glycosulose residues into the corresponding *O*-methyloximes. The glycosulose residues corresponded to 47 and 62 % respectively of the oxidized dextrans, as determined by nitrogen analysis of the corresponding *O*-methyloxime derivatives. When the reaction was performed in half the original amount of phosphoric acid, to obtain a more rigid foam, the proportion of glycosulose residues in the product (oxidized dextran III) was considerably lower (16 %) although the larger amount of sodium nitrite was used. The uronic acid content was the same (~5 %). Apparently, the total amount of glycosulose residues was dependent not only on the amount of sodium nitrite used, but also on the amount of phosphoric acid.

Sugar analysis[13] of the reduced[11] oxidized dextrans revealed the presence of allose, glucose and small amounts of galactose, indicating that the oxidation occurred mainly at C-3 in the glucose residues. The relative amounts of the different glycosulose residues in the oxidized dextrans were determined by ^{13}C NMR spectroscopy of the *O*-methyloximes[9] (Table 1). To evaluate the spectra, it is assumed that the methoximated glycosulose residues in the polymer adopt the same (*E*)-or (*Z*)-configuration as the *O*-methyloxime derivatives of the corresponding methyl α-hexopyranosiduloses. No glycosulose residues containing more than one keto group could be detected. Unlike bromine, sodium nitrite oxidizes dextran

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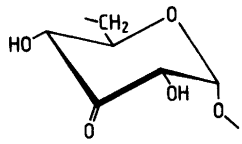

preferentially at C-3, resulting in 3-glycosulose residues (Fig. 1). Small amounts of 4- and traces of 2-glycosulose residues could also be detected in the oxidized dextrans.

A nitric or nitrous acid ester intermediate has been proposed in the oxidation of alcohols and carbohydrates with nitrous acid or nitrogen dioxide,[3,14] similar to the chromate ester intermediate in the chromic acid oxidation of alcohols, where the rate-determining step is the decomposition of this ester.[15] A similar mechanism has been proposed for the oxidation of alcohols with dimethyl sulfoxide–acetic anhydride.[16] Bromine oxidation of secondary alcohols occurs by a different mechanism, whereby the methine hydrogen atom is abstracted by bromine, together with its electron pair, in the rate-determining step.[17] Thus, in α-linked glucopyranosyl units, C-3 is not oxidized by bromine, because of 1,3-diaxial hindrance by the axial aglycone on the methine hydrogen on this carbon.[18] Sodium nitrite in phosphoric acid, however, oxidizes C-3 preferentially, probably because the steric interaction between the axial hydrogen on C-3 and the axial aglycone is relieved.[19]

Experimental. General methods. Solutions were concentrated at reduced pressure below 40 °C. Optical rotations were measured with a Perkin Elmer 141 polarimeter. GLC was performed with a Packard 427 instrument, fitted with a flame-ionisation detector. Separations were performed on a glass column (240×0.15 cm) containing 3 % of OV 225 on Gas Chrom Q (100–120 mesh). ^{13}C NMR spectra were recorded in D_2O on a Jeol FX 90 Q Fourier-transform spectrometer with 16K datapoints (8K zero-filling). The spectral width was 5 kHz and TMS was used as an external standard. Integrations were performed with inverse gated decoupling using a 45° pulse angle, a pulse delay of 4 s, and a pulse repetition time of 4.8 s.

Materials. All chemical reagents used were of reagent grade or better. Sodium nitrite was obtained from E. Merck (Darmstadt, G.F.R.) and methoxylamine hydrochloride from Kodak (Rochester, N.Y. USA). Dextran T 2000 and Superose 6B (20–40 μm) were purchased from Pharmacia (Uppsala, Sweden).

Sodium nitrite oxidations were performed as previously described.[6] The pH was kept at or below 5 throughout the work-up procedure to avoid degradation of the ulose residues.

Methoximations. Methoximations were performed as prevously described.[18] The resulting reaction mixtures were adjusted to pH 7.0, dialysed repeatedly against distilled water, and freeze-dried to give the methoximated oxidized polysaccharides.

Preparation of oxidized dextran I. Dextran T 2000 (5.0 g) was dissolved in ortho-phophoric acid (100 ml) and treated with sodium nitrite (2×5.0 g). The resulting oxidized dextran (4.7 g) had $[\alpha]_{578}^{20}=174°$ (*c* 0.2 water). Sugar analysis of a reduced (sodium borohydride) sample revealed the presence of allose, galactose and glucose in the proportions 4:1:9.

Preparation of oxidized dextran II. Dextran T 2000 (5.0 g) was dissolved in ortho-phosphoric acid (100 ml) and reacted with sodium nitrite (3×5.0 g). The resulting oxidized dextran (4.8 g) had $[\alpha]_{578}^{20}=155°$ (*c* 0.2 water). Sugar analysis of a reduced (sodium borohydride) sample revealed the presence of allose, galactose and glucose in the proportions 4:1:7.

Preparation of oxidized dextran III. Dextran T 2000 (5.0 g) was dissolved in ortho-phosphoric acid (50 ml) and reacted with sodium nitrite (3×5.0 g). The resulting oxidized dextran (4.5 g) had $[\alpha]_{578}^{20}=188°$ (*c* 0.2 water). Sugar analysis of a reduced (sodium borohydride) sample revealed the presence of allose, galactose and glucose, 6:1:43.

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Table 1. Proportions (%) of the sub-units in oxidized dextrans I–III.

Sub-unit	Oxidized dectran		
	I	II	III
2-Glycosulose	2	2	2
3-Glycosulose	31	42	10
4-Glycosulose	13	15	5
Glucose[a]	49	38	76
Uronic acid	5	5	5

[a] Determined by sugar analysis.

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