Evidence for a 'block-type' structure in the main chain of the cell-wall mannan from Candida species PRL 1S20

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Publisher's version / Version de l'éditeur:
https://doi.org/10.1139/v70-032
Canadian Journal of Chemistry, 48, 1, pp. 198-200, 1970-01-01

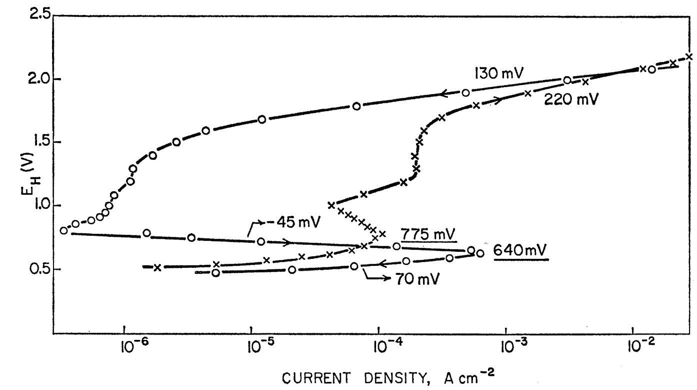

FIG. 2. A steady-state, potentiostatic (point-by-point), current–potential relationship on smooth platinum in 1 N H_2SO_4 plus 2.5×10^{-1} M ethylene glycol under helium atmosphere. ×, Ascending direction of potentials; ○, descending direction potentials. Ascending current reversal point (0.775 V) and descending current reversal point (0.640 V) may be noted.

example suggests that the indiscriminate use of potentiodynamic method for determining the electrode "coverage" does not always yield valid results. It is important that the *origin* of potentiodynamic peaks be established in such studies.

The author is grateful to the referees for helpful comments.

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Evidence for a 'block-type' structure in the main chain of the cell-wall mannan from *Candida* species PRL 1S20[1]

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Received August 8, 1969

Evidence for a 'block-type' structure involving α-(1→3)- and -(1→6)-D-mannopyranosyl units from the mannan in *Candida* species PRL 1S20 is presented.

Canadian Journal of Chemistry, **48**, 198 (1970)

In a previous publication the branched-chain mannan obtained from cells of *Candida* species PRL 1S20 by extraction with hot aqueous alkali has been shown to contain a main chain not previously found in other yeast mannans (1). The main chain was obtained from the mannan by a Smith degradation incorporating mild hydrolytic conditions. Methylation-fragmentation – gas–liquid chromatography (g.l.c.) analysis gave the methyl glycosides of 2,3,4,6-tetra-*O*-, 2,3,4-, and 2,4,6-tri-*O*-methylmannose. No methyl di-*O*-methyl-mannosides were detected. After corrections for the response of the g.l.c. detector are made, a respective 1:4.3:7.0 molar ratio of the fragments can be calculated. This ratio indicates a linear mannan with an average size of about 12 units and its specific rotation of +55° shows a preponderance of

[1]Issued as NRCC No. 10960

3-*O*-linked α-D-mannopyranose units, although the configurations of the 6-*O*- substituted units are uncertain. (It appears that the 3-*O*- and 6-*O*-substituted units occur in the same molecule since 2 successive Smith degradations of the *Candida* PRL 1S20 mannan gave a mannosyl glycerol (1).) This type of main-chain differs from those of the more common α-(1 → 6)-D-mannopyranose (1–6) and α-(1 → 3)-D-mannopyranose types (7, 8) and the linear mannan containing alternating β-(1 → 3)- and β-(1 → 4)-linked D-mannopyranose units (9).

In order to determine the sequence of (1 → 6)- and (1 → 3)- linkages in the *Candida* PRL 1S20 mannan main-chain, the polyalcohol derived by successive treatments of the undegraded mannan with sodium periodate and sodium borohydride was partially cleaved by acetolysis. This procedure splits (1 → 6)-linkages in mannans in preference to (1 → 2)- and (1 → 3)-linkages (10). Deacetylation of the products and paper chromatography showed mannose and oligosaccharides which appeared to have a maximum length of 5 units. These were isolated by cellulose column chromatography (yields in Table 1).

TABLE 1

Yields and specific rotations of fragments obtained following partial acetolysis of *C. Candida* PRL 1S20 mannan polyalcohol

Fragment obtained following partial acetolysis of *Candida* PRL 1S20 mannan polyalcohol	Yield (%) based on polyalcohol	Specific rotation (°), 0.1% solution at 25 °C
Mannose	2	—
Disaccharide	4	+61
Trisaccharide	3	+62
Tetrasaccharide	3	+79
Pentasaccharide	1	+88

Methylation-fragmentation – g.l.c. analysis of each oligosaccharide gave methyl 2,3,4,6-tetra-*O*- and methyl 2,4,6-tri-*O*-methylmannoside indicating 3-*O*-linked pyranose structures. The chain length of each oligosaccharide was found as follows. Their reducing ends were converted to 2-*O*-linked-D-arabinitol units by successive treatments with lead tetraacetate and sodium borohydride (11). The resulting polyols were oxidized with sodium periodate and the amounts of formaldehyde formed, as estimated by the chromotropic acid method, were in accord with the chain lengths suggested by the paper chromatographic mobility of the parent mannose oligosaccharides.

The specific rotations of the di-, tri-, tetra-, and pentasaccharide (Table 1) confirm that the 3-*O*-linkages have an α-configuration. Isolation of these fragments and comparison with the ratio of 3-*O*- to 6-*O*-linked D-mannopyranose units in the mannan shows that the main chain of *Candida* PRL 1S20 mannan does not contain 3-*O*-α- and 6-*O*-linked units evenly distributed along its length. Rather, a 'block-type' structure containing up to four consecutive α-(1 → 3)-linkages is indicated, which contrasts with the regular alternating structure of the β-(1 → 3)-, β-(1 → 4)-linked mannan (9).

Experimental

Conversion of Candida PRL 1S20 Mannan to its Polyalcohol

The mannan (1.50 g) was dissolved in water (25 ml) and sodium periodate (3.5 g) added, precautions being taken to prevent overheating. After 2 days, excess acetic acid (200 ml) was added and the precipitate of polyaldehyde which formed was removed by filtration; yield 1.38 g. The polyaldehyde was shaken in a solution of sodium borohydride (0.4 g) in water (50 ml) for 3 h. Excess reagent was then destroyed by addition of acetic acid, and the solution treated with a mixture of Amberlite IR120 (H^+ form) and Dowex 1 resin (bicarbonate form). The solution obtained after filtration was lyophilized to give a polyalcohol (1.21 g), which had the fluffy state required for the following partial acetolysis procedure.

Partial Acetolysis of Candida PRL 1S20 Polyalcohol

The polyalcohol (1.21 g) was partially acetolyzed (10) for 24 h with the reagent proportions used by Lee and Ballou (12). The product was deacetylated and the free sugars corresponded on a paper chromatogram (solvent: *n*-butanol – ethanol – water 3:2:2 v/v/v; spray: ammoniacal silver nitrate) to glycerol, mannose and 3-*O*-α-D-mannopyranosyl-D-mannose and higher oligosaccharides. The higher oligosaccharides had chromatographic rates, relative to lactose, of 1.1, 0.93, and 0.54, which were identical to those of α-(1 → 2)-linked D-mannopyranose tri-, tetra-, and pentasaccharides, respectively.

Isolation and Identification of Oligosaccharide Fragments

The product obtained by partial acetolysis of the *Candida* PRL 1S20 mannan polyalcohol was fractionated on a cellulose column using acetone–water mixtures in proportions described in a previous publication (1). The yields and specific rotations of the fragments are presented in Table 1. Hydrolysis of each fraction gave mannose only.

The di-, tri-, tetra-, and pentasaccharides were each partially methylated by the Haworth technique (13), care being taken to maintain the p*H* of the solution at 7–8 in the early stages of the reaction. After heating at 100° for 3 h, the alkali was neutralized and each solution extracted continuously with chloroform. The extracted products were completely methylated by the Kuhn procedure (14), each permethylated product showing an absence of O—H absorption in the infrared at 3600 cm^{-1}. The methylated materials were converted to methyl *O*-methyl mannosides and their trimethylsilyl derivatives, which were analysed by g.l.c. using known standards (15). From each oligosaccharide, the methyl glycosides of only 2,3,4,6-tetra-*O*- and 2,4,6-tri-*O*-methylmannose could be detected.

Each oligosaccharide was successively treated with lead tetraacetate and excess sodium borohydride (11), converting their 3-*O*-linked D-mannopyranose reducing end-units to 2-*O*-linked D-arabinitol units. In a typical reaction the 3-*O*-linked D-mannopyranose oligosaccharide (10 mg) was dissolved in water (0.1 ml) and acetic acid (1 ml) added. Molar equivalents (1.2) of a 1% lead tetraacetate in acetic acid were added and after 10 min it was followed by 1.1 molar equivalents of oxalic acid in acetic acid. After 1 h the precipitate of lead oxalate was filtered off and the filtrate evaporated to dryness. Sodium borohydride (10 mg) was added and in 1 h the reagent was destroyed with excess acetic acid. The solution was shaken with Amberlite IR120 to remove cations and was then filtered and the filtrate evaporated to dryness. Repeated addition of methanol followed by evaporation removed boric acid. Traces of inorganic material were removed with an aqueous slurry of Amberlite IR120 (H^+ form) and Dowex 1-X8 (bicarbonate form). The slurry was filtered and the solution evaporated. The product gave a single spot on a paper chromatogram (solvent: *n*-butanol – ethanol – water 40:11:19 v/v/v spray ammoniacal silver nitrate). Each arabinitol derivative thus obtained was treated with excess sodium periodate for 1 h and the amounts of formaldehyde produced determined by a chromotropic acid method used for estimation of glycerol (16). The yields of formaldehyde in mole/mole were as follows: trisaccharide, 0.86; tetrasaccharide, 0.83; pentasaccharide, 0.88.

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