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# Note

## Structure of β-D-glucans from *Fusarium oxysporum* *

Maud Bruneteau, Jacky Perret, Philippe Rouhier and Georges Michel
*Laboratoire de Biochimie Microbienne, Université Claude Bernard-Lyon I, F-69622 Villeurbanne (France)*

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D-Glucans have been found as common polymers of the fungal cell wall in basidiomycetes, ascomycetes[1], and oomycetes[2]. In recent studies, we isolated several D-glucans from cell walls of *Phytophthora parasitica*, a phytopathogenic fungus of carnation[3]. They consist of a mixture of linear (1 → 3)-β-D-glucans with various (1 → 6)-branched oligosaccharide chains, the length of these chains being modulated by the culture conditions[4]. In all fungal polysaccharides previously described, only mono- and di-saccharide branches were observed, while tri- or tetra-saccharide branches were found in *P. parasitica*. We report herein the structure of *Fusarium oxysporum* β-D-glucans with a view to compare the structure of the cell wall glucans of *F. oxysporum* with those of β-D-glucans of *P. parasitica*.

The cell walls from *F. oxysporum* were prepared as previously reported[3], and obtained in a yield of 2–5% of the fresh weight of the mycelium. The material released by successive hot water and sodium hydroxide extractions accounted for 25% of the walls. The alkali extract was fractionated by column chromatography on DEAE-cellulose; the analysis of the first fraction, eluted with 10 mM potassium phosphate buffer, showed that D-glucose was the unique component.

Gel filtration of these glucans on Sepharose CL-4B indicated a range of molecular weights from $2 \times 10^6$ to $2 \times 10^4$ with a preponderance of material of high molecular weight (yield, 58%).

The glucans were twice methylated by the Hakomori method[5]. After hydrolysis, reduction and acetylation, GLC of the alditol acetates from fully methylated glucans showed three peaks corresponding to 2,3,4,6-tetra-*O*-methyl, 2,4,6-tri-*O*-methyl, and 2,4-di-*O*-methyl derivatives in a molar ratio of 1.0:5.0:0.9 (Table I). These results indicated a (1 → 3)-linked backbone with (1 → 6)-linked branches.

*Correspondence to*: Dr. M. Bruneteau, Laboratoire de Biochimie Microbienne, Université Claude Bernard-Lyon I, F-69622 Villeurbanne Cedex, France.
* Dedicated to Professor Jean Montreuil.

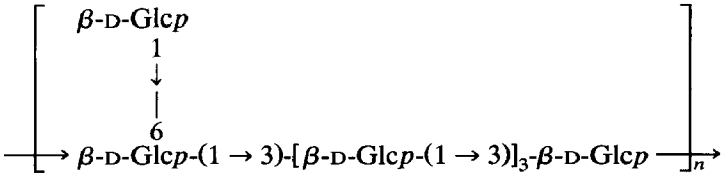
TABLE I

Molar ratios of the hydrolysis products of methylated native glucans and Smith degraded glucans

| *O*-Methyl-D-glucose | Linkage indicated | Molar ratios of glucans | |
|---|---|---|---|
| | | Native | Smith degraded |
| 2,3,4,6-Tetra- | Glc-(1 → | 1.0 | 1.0 |
| 2,4,6-Tri- | → 3)-Glc-(1 → | 5.0 | 18.0 |
| 2,4-Di- | → 3)-Glc-(1 → 6 ↑ | 0.9 | |

After acetolysis, gel filtration on Sephadex G-15 gave only two fractions. The highest-molecular-weight fraction was eluted with the void volume, and its structure was analyzed by methylation, hydrolysis, reduction, acetylation, and GLC of derivatives. The identification of 2,3,4,6-tetra-*O*-methyl and 2,4,6-tri-*O*-methyl derivatives agrees with a (1 → 3)-linked oligosaccharide resulting from complete cleavage off of the branched chains and probably from some cleavage of (1 → 3) bonds of the backbone. The second peak was composed of only glucose monomers, suggesting the presence of single D-glucosyl groups as side chains attached at O-6 of some of the main-chain units.

The glucans were submitted to periodate oxidation, borohydride reduction, and hydrolysis under mild conditions by heating with 0.5 M trifluoroacetic acid at 20° for 15 h (Smith degradation)[6]. The Smith-degraded polysaccharide was obtained as an insoluble product separated by centrifugation. Methylation analysis of this polymer gave 2,4,6-tri-*O*-methyl and 2,3,4,6-tetra-*O*-methyl derivatives in a molar ratio of 18:1 (Table I); this result was in agreement with a (1 → 3)-linked backbone chain. Detection of only D-glycerol but not of D-glucosylglycerol in the soluble fraction confirmed the presence of single glucosyl groups as side chains. The β configuration of the D-glucosyl groups was evidenced by the presence of characteristic signals in the $^{13}C$ NMR spectrum, which was similar to that of lentinan[7]. The results of methylation analysis, acetolysis and Smith degradation suggest structure **1** for the D-glucans of *F. oxysporum*.




**1**

*F. oxysporum* produce β-D-glucans of the same type as those of *P. parasitica* Dastur with a straight chain having (1 → 3)-linked β-D-glucose residues, substituted with branched chains. Some differences were found in the structure of the branched chains. The D-glucans of *P. parasitica* have a more complex structure, the branched chains containing either one D-glucosyl group or di-, tri-, or tetra-D-

glucose side chains, whereas only monosaccharide chains were found in *F. oxysporum*. The D-glucans of *F. oxysporum* were structuraly similar to the D-glucans obtained from basidiomycetes[1] and ascomycetes[2] which have a moderate degree of branching of one out of five D-glucose residues.

## EXPERIMENTAL

*Materials.*—The isolate of *F. oxysporum* from the fungal culture collection of INRA Montfavet was grown on unshaken, synthetic liquid medium[8] during 3 weeks at 24° under 16 h of light by day. The mycelium was separated from the medium by centrifugation. DEAE-cellulose was purchased from Whatman Inc.; Sepharose CL-4B and standard dextrans were obtained from Pharmacia LKB. Other chemicals were standard chemical products.

*General analytical methods.*—Total content in glucose was determined by a colorimetric assay according to Fischer and Zapf[9].

For GLC, glucose and methylated derivatives were converted into the corresponding alditol acetates[10]. GLC was carried out on an Intersmat apparatus (model 120FL), fitted with a glass capillary SP 2380 column (0.25 mm × 20 m). GLC–MS was performed on a UG Micromass 305 apparatus equipped with a capillary column BP1 (0.25 mm × 60 m) and with a temperature programm (120 to 160°, rate 5°/min; and 160 to 280°, rate 2°/min). Mass spectra were taken at an ion energy of 70 eV, a current intensity of 200 μA, and a temperature of 180°.

*Extraction of glucans.*—Glucans were isolated from mycelial walls. The cell walls from *F. oxysporum* were prepared as previously reported[3]. They were extracted with hot water. The insoluble residue was suspended in M NaOH for 2 h at 60° and then centrifuged[11]. The supernatant solution was neutralized with acetic acid and then dialyzed against distilled water overnight. The nondialyzable fraction was lyophilized. The resulting material was dissolved in 0.01 M sodium phosphate buffer, pH 7.0, and fractionated by column chromatography on D52 DEAE-cellulose, equilibrated with the same buffer. The column was first eluted with the same buffer, and then with a linear gradient of NaCl (0–1 M) in the same buffer. Each fraction was dialyzed and subjected to chromatography on a column of Sephadex G-25 eluted with water.

*Determination of molecular weight.*—A solution of glucans (3 mg) in distilled water (0.5 mL) was applied to a column (1.6 × 80 cm) of Sepharose CL-4B. The column was equilibrated and eluted with distilled water at a flow rate of 10 mL/h and the effluent was collected in 4-mL fractions. The carbohydrate content of each fraction was determined with the anthrone reagent[12]. The column was calibrated with standard dextrans (mol wt $10^4$–$2 \times 10^6$).

*Methylation analysis.*—A sample (1–5 mg) of polysaccharide dissolved in $Me_2SO$ (1 mL) was methylated by the method of Hakomori[5]. After methylation, the mixture was dialyzed and the nondialyzable fraction concentrated to dryness. The permethylated polysaccharide was purified by elution from a LH-20 column with

2:1 EtOH–$CHCl_3$, dried, and hydrolyzed by heating in 85% formic acid (2 mL) at 100° for 5 h and, after removal of the formic acid, in M trifluoroacetic acid (2 mL) under the same conditions[11]. After evaporation to dryness, the methylated sugars were reduced with $NaBD_4$, acetylated with acetic anhydride, and analyzed as alditol acetates by GLC[13]. The identification of methylated sugars was performed by GLC–MS[14].

*Smith degradation.*—A sample (10 mg) was oxidized with 0.05 M $NaIO_4$ (10 mL) at 4° in the dark during one week. The oxidation was stopped by addition of 1,2-ethanediol and the solution dialyzed against distilled water. The dialyzed material was reduced with $NaBH_4$ for 15 h, neutralized with 50% acetic acid, dialyzed, and partially hydrolyzed in 0.5 M trifluoracetic acid for 15 h at 20° (ref. 6). The undissolved material was removed by centrifugation. The supernatant was neutralized by evaporation of excess acid and fractionated by chromatography on Sephadex G-15.

*Acetolysis.*—Acetolysis of glucans (10 mg) was performed according to Dubourdieu et al.[15] in 10:10:1 acetic anhydride–acetic acid–$H_2SO_4$ (10 mL) as previously described[16]. After acetolysis, the sample was fractionated by column chromatography on Sephadex G-15.

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