Identification of $\beta 1\rightarrow 4$ Glucan Chains as Part of a Fraction of Slime Synthesized within the Dictyosomes of Maize Root Caps

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Summary

The protective polysaccharides synthesized by the outer root-cap cells of maize have been prepared in radioactive and non-radioactive form and studied using the techniques of trans-elimination, gel filtration and partial hydrolysis under acid and alkaline conditions. The results indicate that the slime consists of a central $\beta 1\rightarrow 4$ linked glucan rendered soluble by a coating of hydrophilic polysaccharides linked both covalently and non-covalently. The covalently-linked polysaccharide is relatively rich in galacturonic acid and fucose in regions near the central glucan. It is likely that the synthesis of the slime, including the glucan component, takes place within the dictyosome sacs and vesicles and this has important consequences for ideas on the sites of $\beta 1\rightarrow 4$ glucan synthesis within plant cells.

1. Introduction

Maize outer root-cap cells synthesize a polysaccharide whose function is thought to be protective. The structure of the slime has been studied in detail and it contains a high proportion of L-fucose (Harris and Northcote 1970) which occurs only in trace amounts in polysaccharides found elsewhere in the maize seedling (Wright and Northcote 1974). The slime contains galacturonic acid (Jones and Morré 1967, Harris and Northcote 1970) and it can be separated by glass-fibre paper electrophoresis into neutral, weakly-acidic and acidic polymers. The neutral polymers always contained glucose and in some preparations mainly this sugar (Wright and Northcote 1974, Wright 1975). The acidic polymers underwent transelimination under alkaline conditions (Wright and Northcote 1974). These properties of

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maize slime resembled those of pectins, although in slime a good deal of the galacturonic acid was glycosidically attached to glucose (Wright and Northcote 1975).

The fragments of slime which were studied previously were separated electrophoretically according to their charge. In the experiments reported here we have combined this technique with that of gel-filtration chromatography to examine the fragments of radioactive slime which were released by t-elimination. We also report observations made with preparations of non-radioactive slime which relate to the overall structure of the polysaccharide complex, and which have important consequences for ideas on the sites of β1 → 4 glucan synthesis within the plant cell.

2. Materials and Methods

2.1. Production of Radioactively Labelled Slime

Seeds of Zea mays L cv Caldera 535 were sterilized and germinated by method (2) of Wright (1975) including treatment with 0.1% HgCl₂ (20 minutes); this modification of previous procedures was necessary since recent batches of seeds were supplied dressed with a fungicide which, using method (1) was less effective. Slime labelled by feeding D-[U-14C] glucose (Wright and Northcote 1974) was stored frozen in solution rather than freeze-dried. Slime labelled with L-[1-3H] fucose was prepared after feeding roots with this precursor for 3 hours (Wright, Northcote, and Davy 1976).

2.2. Gel Permeation Chromatography of Radioactive Slime

The column packing material and the 7 mm diameter disposable columns which were used with P-100 and P-200 gels were supplied by Bio-Rad Laboratories Ltd., Bromley, Kent BR4 1T3. The bed height was 150 mm in all cases, and the column used for P-4 had a diameter of 15 mm. An LKB 12,000 Varioperpex pump was used to maintain a regular flow of water through the columns at their optimal flow rates (P-4, 80 ml/h; P-100, P-200, 7.3 ml/h) and fractions were collected on an LKB Ultrorac 7,000 fraction collector after passing through an LKB 8,300 Uvicord II fitted with a 280 nm filter. The radioactivity in the eluate was determined by mixing (1:20) a suitable volume from each fraction with Triton/toluene scintillant (6 g 2,5-diphenyloxazole, 75 mg 1,4-di[2-(5-phenyloxazolyl)] benzene, 750 ml Triton X-100, 1500 ml toluene).

2.3. Analytical Methods

Transelimination (the degradation of an esterified 1 → 4 galacturonan at pH values of 6.8 or above due to trans-elimination of a proton at C-5 and the breakage of the glycosidic bond) was brought about by mixing equal volumes of the solution with 0.2 M Na₂HPO₄ (pH 9.2) and heating in a sealed vial at 100°C for 60 hours. This pH value was more effective for the reaction than pH 6.8 (Barrett and Northcote 1965, Wright and Northcote 1974).

The positions of radioactive materials after electrophoresis on glass-fibre paper at pH 6.5 (100 ml of pyridine, 3 ml of acetic acid made up to 1 l with water) (Wright 1975) was determined by cutting the dried paper with a scalpel into 40 × 10 mm strips, with the long edge perpendicular to the direction of the run. The strips were placed in scintillation vials, wetted with 1 ml scintillation fluid [8.75 g 2,5-diphenyloxazole, 125 mg 1,4-bis-(5-phenyloxazol-2-yl)benzene in 2.5 l sulphur-free toluene] and the radioactivity determined using a Nuclear-Chicago Unilux Scintillation Counter. Neutral material which moved