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Note

Isolation of an electrophoretically homogeneous glucomannan, using glutaraldehyde-insolubilised concanavalin A

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The reaction between concanavalin A, a phytohaemagglutinin isolated from jack bean (*Canavalia ensiformis* L), and various polysaccharides, lipopolysaccharides, and glycoproteins, to form insoluble complexes has recently received considerable attention[1–5] The interaction depends not only on the presence of α-D-mannopyranosyl, α-D-glucopyranosyl, 2-acetamido-2-deoxy-α-D-glucopyranosyl, and β-D-fructofuranosyl end-residues in the polymer but also on such factors as the degree of branching and the molecular weight of the polysaccharides[6–9].

Specific adsorbents for carbohydrates can be obtained by attachment of concanavalin A to an inert agarose matrix, activated by cyanogen bromide[10 11] or by modification with L-leucine *N*-carboxyanhydride[11] or glutaraldehyde[12] We now

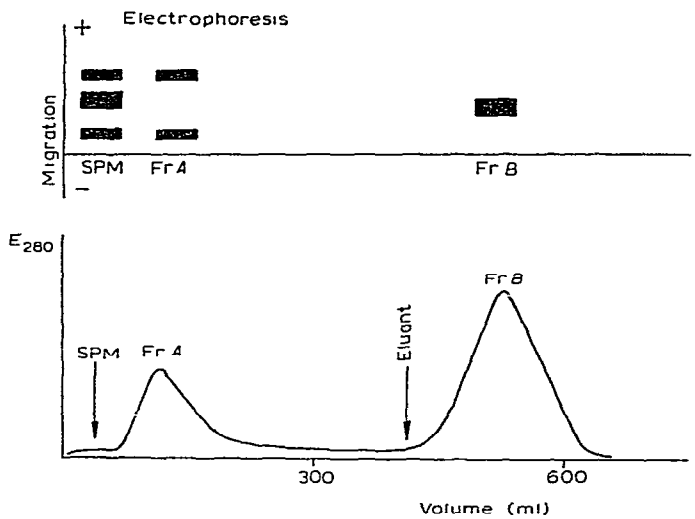

Fig. 1 Fractionation of a water-soluble polysaccharide mixture from *Arum orientale*, coloured with a reactive dye, on a column of glutaraldehyde-insolubilised concanavalin A The upper part of the figure shows the electrophoresis (on cellulose acetate strips) of the starting polysaccharide mixture (SPM), fraction *A*, and fraction *B*

report on the use of concanavalin A cross-linked with glutaraldehyde for the specific isolation of a glucomannan from the tubers of *Arum orientale* (L)

Column chromatography of the water-soluble polysaccharide mixture from the tubers of *Arum orientale*, pre-coloured with a reactive dye (Procion blue M 3G), on a column of glutaraldehyde-insolubilized concanavalin A showed (Fig 1) that only fraction *B* of the polysaccharide was bound specifically, whereas the remaining components (Fraction *A*) were eluted Fraction *B* was readily displaced from the column by methyl α-D-mannopyranoside, which also strongly inhibited the concanavalin A–polysaccharide interaction[7 13]. It was thus possible to carry out a fractionation on the unmodified (*i e*, colourless) polysaccharide mixture, and the fraction *B* so obtained was used for further investigations.

Fraction *B* was proved to be homogeneous by electrophoresis on cellulose acetate strips (Fig 1) in two different buffers (*J* and *K*) In contrast, fraction *A* was heterogeneous and consisted of two polysaccharides (Fig 1)

Complete acid hydrolysis of fraction *B* gave D-mannose, D-glucose, and traces of uronic acid At 5°, fraction *B* consumed 1 0 mole of periodate per hexose residue. Complete oxidation of the glucomannan was achieved after 6 days, and reduction of the resulting polyaldehyde with borohydride, followed by acid hydrolysis of the polyalcohol, gave glycerol (trace), erythritol, and D-mannose (trace)

The I R spectrum of fraction *B* showed absorptions at 1730 and 1248 (COOH), and 894, 875, and 800 cm^{-1}, variously indicative[14] of β-D-glucopyranose and β-D-mannopyranose residues

The above data indicate that fraction *B* is a glucomannan containing β-(1→4)-D-glucosyl linkages, hexose residues in the β-D-pyranoid form, and some branching.

EXPERIMENTAL

General methods — Protein concentrations were determined by the biuret[15] and Lowry–Folin[16] methods Fractions were tested for carbohydrate by the phenol–sulphuric acid method[17] at 490 nm Paper chromatography (p c) was performed on Whatman No 1 and 3MM papers, using *A*, acetone–1-butanol–water (7 2 1), *B*, ethanol–1–butanol–water (5 4 1), *C*, ethyl acetate–pyridine–water (8 2·1), and *D*, *tert*-pentyl alcohol–propanol–water (40 10 15) Sugars were detected with aniline hydrogen phthalate (*G*), and higher alcohols, glycerol, and erythritol with sodium metaperiodate-benzidine (*H*) Thin-layer chromatography (t l c) was performed on silica gel G impregnated with 0 2M sodium dihydrogen phosphate by using *E*, ethyl acetate–2-propanol–water (8 2 1), and *F*, acetone–water–chloroform–methanol (8 5:1 1), and detection with naphthoresorcinol[18] (*I*)

Concanavalin A was prepared from jack-bean meal (*Canavalia ensiformis* L) by absorption on Sephadex G-100 and subsequent elution with D-glucose[19]

Polymerization of concanavalin A with glutaraldehyde — Concanavalin A, cross-linked with glutaraldehyde in admixture with Biogel P-10, was prepared and used for column chromatography as described by Donnelly and Goldstein[12]. The column was washed with M sodium chloride before use.

Binding and displacement of the glucomannan — (*a*) The crude, water-soluble polysaccharide mixture (80 mg) obtained[18] from the tubers of *Arum orientale* and pre-coloured with Procion blue M 3G (I.C I , Deutschland GmbH) was dissolved in M sodium chloride and applied to the column[12] described above The column was washed with M sodium chloride until the starting extinction was obtained, and then was eluted with M sodium chloride containing 0.1M methyl α-D-mannopyranoside Fractions (~6 ml) were collected automatically and their extinctions determined at 280 nm The contents of the fractions corresponding to peaks *A* and *B* (Fig 1), respectively, were combined, dialysed against water, and freeze-dried

(*b*) The crude, water-soluble polysaccharide mixture (80 mg) dissolved in M sodium chloride was applied to the column and eluted as described above The contents of the fractions corresponding to peaks *A* and *B* were combined and isolated as described above

Electrophoresis studies. — The Procion dye complexes from fractions *A* and *B* were subjected to electrophoresis [0 05M borate buffer (*J*) and 0 1M borax–sodium chloride buffer (*K*), 270 volts, 4–5 min] on cellulose acetate strips (25 × 140 mm) by the Dudman–Bishop method[20]

Hydrolysis of fraction B — A sample (40 mg) of fraction *B* in 5% sulphuric acid (4 ml) was heated at 100° for 24 h After neutralization ($BaCO_3$), the hydrolysate was deionized with Duolite A-4 and Amberlite IR-120 resins and evaporated The syrupy residue was analyzed by p c (solvents *A* and *B*) and t l c (solvents *E* and *F*), with detection by reagents *G* and *I*

Periodate oxidation of fraction B — To a portion (40 mg) of fraction *B* dissolved in water (30 ml), 0 01M sodium periodate (90 ml) was added, and the solution was kept at 5° in the dark The periodate consumption was determined by the Fleury–Lange method[21], and formic acid was measured by titration with 0 01M sodium hydroxide after reduction of the excess periodate with ethylene glycol. The resulting polyaldehyde was dialysed against water and then reduced with sodium borohydride (700 mg) at room temperature for 2 h with continuous stirring The polyalcohol was dialysed against water, isolated by freeze-drying, and hydrolysed with 0 5M sulphuric acid in an ampoule at 100° for 10 h After neutralization ($BaCO_3$), the hydrolysate was deionised (see above) and evaporated The syrupy residue was analyzed by p c (solvents *C* and *D*) with detection by reagent *H* The presence of mannose was proved by p c and t l c , as described above

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