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Note

A new method for the analysis of laminarins and for preparative-scale fractionation of their components

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Detailed structural studies of certain laminarins have been hampered by the lack of a method for separating the mannitol-terminated chains (M-chains) from the glucose-terminated chains (G-chains). Lewis and Smith[1] described a fractionation of laminarin from *Laminaria hyperborea* (*L. cloustoni*) into three components by electrophoresis on glass-fibre paper, using borate buffer at an alkaline pH. Using bromine oxidation, followed by chromatography on an anion-exchange resin, the components of laminarin have been separated[2] into "laminaric acid" (from the oxidised G-chains) and mannitol-terminated chains. More recently, laminarin from *Laminaria hyperborea* has been fractionated[3] by differential solubility into branched and unbranched components. These three methods do not, however, provide a means of separating the two main components of laminarin (M-chains and G-chains) in an undegraded form.

In the present work, a new method for fractionating laminarin into its components on a preparative scale is described. Since the method is quantitative, it can also be used to determine the ratio of M-chains to G-chains. Further analytical uses of the method are at present being studied.

EXPERIMENTAL

In this work, insoluble laminarin[4] (B.B.2) from *Laminaria hyperborea* was used.

Methods. — Assays for total carbohydrate were carried out by the phenol–sulphuric acid method[5].

Paper chromatograms were developed on Whatman No 1 paper, using butanone–acetic acid–water (9:1:1) saturated with boric acid, and ethyl acetate–pyridine–water (10:4:3). Periodate–permanganate spray reagent[6] and a silver nitrate[7] dip were used.

Total, acid hydrolysates were prepared by heating a 1% solution of the polysaccharide with M H_2SO_4 for 2 h at 98°; for partial, acid hydrolysis, 0.17M H_2SO_4 was used at 98° for 1 h; barium carbonate was used for neutralisation.

Periodate oxidations were carried out at 2°, as described by Anderson *et al.*[4]. Reducing power of the fractions was measured by a modification[8] of the Nelson method[9].

Preparation of DEAE-Sephadex–molybdate column. — DEAE-Sephadex (A-50) (1.5 g) was heated at 98° for 1 h with three changes of 0.01M sodium molybdate buffer (pH 5.0; 250 ml each change). The gel was then washed with freshly distilled water and packed as a slurry in water. This size of column can readily fractionate quantities of laminarin ranging from 10–200 mg. Larger columns can be used to fractionate gram quantities, but care has to be taken with insoluble laminarins to see that the total time for elution of the column does not exceed 5–6 h; otherwise, low yields are obtained due to precipitation. DEAE-Cellulose, treated as described above, gave similar results. In a typical separation, laminarin (100 mg) dissolved in water (5 ml) was loaded on to the column. Fractions (5 ml) were collected; the column was eluted with water (100 ml) followed by 0.25M sodium chloride (100 ml).

RESULTS

The results of a typical run are shown in Fig. 1. In this experiment, laminarin (72.0 mg; as estimated by the phenol–sulphuric acid method[5]) was loaded on to the column and eluted as described above. Phenol–sulphuric acid analysis of the column fractions indicated the presence of fractions *A* (33.2 mg) and *B* (36.5 mg), corresponding to an overall recovery of 97%.

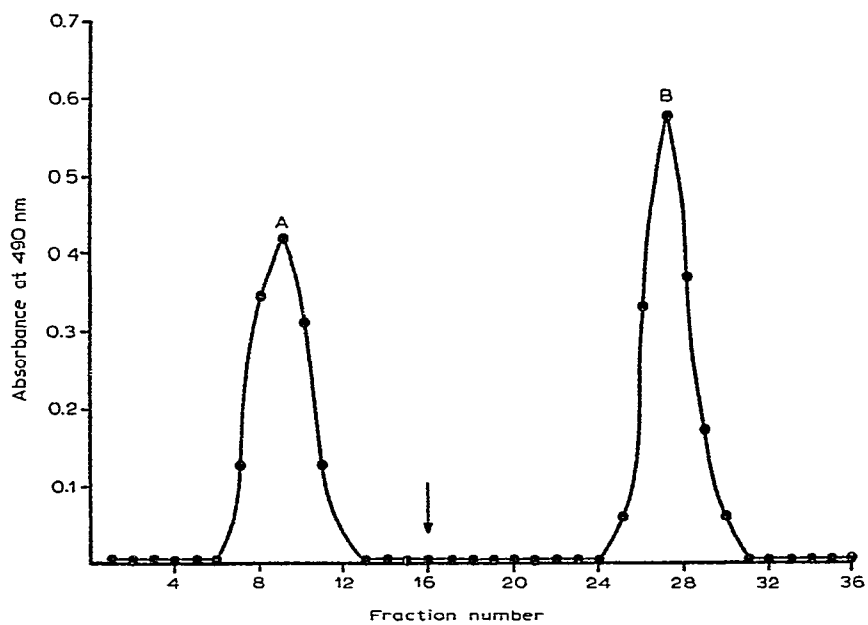

Fig. 1. Separation of the components of laminarin on DEAE-Sephadex–molybdate: —●—, phenol–sulphuric acid reaction. The vertical arrow indicates the change from water to 0.25M sodium chloride.

Fraction *A* yielded only glucose on total, acid hydrolysis; it had a relatively high reducing-power, and when oxidised by sodium metaperiodate at 2° yielded negligible amounts of formaldehyde. This is in contrast to fraction *B*, which yielded glucose and mannitol on total, acid hydrolysis, had a negligible reducing-power, and readily yielded formaldehyde on periodate oxidation at 2° (41 mmol of formaldehyde per "anhydrohexose" unit, equivalent to an average degree of polymerisation of 24 glucose residues).

Furthermore, when a partial, acid hydrolysate of fraction *A* was eluted from a column of DEAE-Sephadex–molybdate, only one peak was obtained. When a partial, acid hydrolysate of fraction *B* was eluted from DEAE-Sephadex–molybdate, two fractions were obtained; one corresponded to glucose and glucose-terminated oligosaccharides (which are readily eluted from the column with water), and the other to mannitol and mannitol-terminated oligosaccharides (which require salt solution for elution). These results indicate that a clear-cut separation of the two components of laminarin had been achieved.

Similar results for a soluble laminarin from *L. digitata* have recently been obtained by Mr. Brian Monaghan in this laboratory, using a DEAE-cellulose–molybdate column.

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