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Note

Column chromatographic separation and quantitation of α-linked glucose oligosaccharides

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Since Khym and Zill[1] initially reported on the chromatographic separation of neutral sugars in borate buffer medium, several investigations with a variety of ion-exchange resins and modifications of the procedures have been reported[2–5]. Several systems have also been described for the automated column-chromatographic analysis of saccharides[6–9].

During our immunochemical studies on dextrans[10,11] an attempt was made to separate and determine α-linked glucose oligosaccharides by using an automated system with an anion-exchange resin and borate buffers. This communication describes the results of the experiments.

EXPERIMENTAL

Materials

Kojibiose (O-α-D-glucopyranosyl-(1→2)-D-glucose), nigerose (O-α-D-glucopyranosyl-(1→3)-D-glucose), maltose (O-α-D-glucopyranosyl-(1→4)-D-glucose), isomaltose (O-α-D-glucopyranosyl-(1→6)-D-glucose), isomaltosyl oligosaccharides up to the hexamer, kojibiitol, nigeritol, maltitol and isomaltitol were the samples described previously[10]. Isomaltoheptaose was prepared as described by Schlossman and Kabat[12], and had an $[\alpha]_D^{20}$ value of +156° (ref. 12, +164°). The ratio of glucose contents of this sample before and after reduction with sodium tetrahydroborate was shown to be 7.00:6.03 by the method of Sakakibara *et al.*[10]. Methyl α-D-glucoside was the commercial product (Pierce, Rockford, Ill., U.S.A.). Dextran NRRL B1355 S was a generous gift from Dr. A. R. Jeanes, Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, Ill., U.S.A.

The borate solutions used for the column procedure were prepared from analytical-grade boric acid. The concentrations of buffer were: (1) 0.13 *M* boric acid, pH 7.5, (2) 0.25 *M* boric acid, pH 9.0, and (3) 0.35 *M* boric acid, pH 9.6. The pH values were adjusted with saturated sodium hydroxide solution.

The anion-exchange resin used was JEOL resin LCR-3 (Japan Electronic Optical Laboratory, Tokyo, Japan): this was washed alternately several times with 2 *M* hydrochloric acid and 2 *M* sodium hydroxide. After preparation of a column, the resin was activated with 0.5 *M* potassium tetraborate and then with buffer (1).

Methods

The sugars were determined colorimetrically after reaction at 95° with 0.15% orcinol in concentrated sulphuric acid.

A JEOL Liquid Chromatograph Model JLC-3BC was used for the automated saccharide-analysis system. The pumps were set to deliver buffers at 0.51 ml/min and orcinol reagent at 0.94 ml/min, and the column size was 0.8 × 15 cm. Chromatography was carried out under two sets of conditions: under conditions (i), the column temperature was 55° and buffers (1), (2) and (3) were delivered for 100, 40 and 220 min, respectively; under conditions (ii), the temperature was 65° and buffers (1), (2) and (3) were delivered for 60, 70 and 230 min, respectively.

Acetolysis of dextran and de-acetylation of the products were carried out as described previously[13]. Reduction of the de-acetylated materials was also carried out as described previously[10].

RESULTS AND DISCUSSION

Resolution of α-linked glucose oligosaccharides and reduced disaccharides

Various α-linked glucose oligosaccharides and reduced samples were subjected to column chromatography. Fig. 1 shows the separation of isomaltosyl oligosaccha-

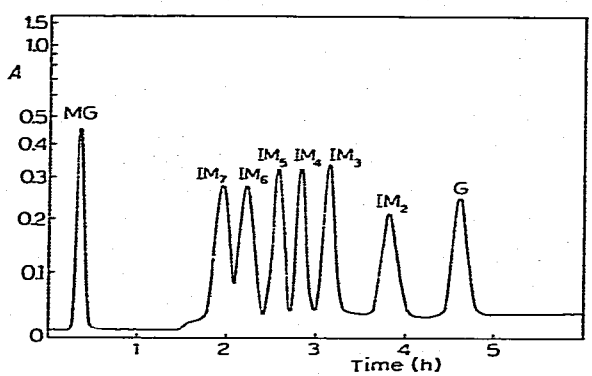

Fig. 1. Chromatographic separation, under conditions (ii), of a mixture containing 0.25 μmole of D-glucose (G), 0.12 μmole of isomaltose (IM_2), 0.08 μmole of isomaltotriose (IM_3), 0.06 μmole of isomaltotetraose (IM_4), 0.05 μmole of isomaltopentaose (IM_5), 0.04 μmole of isomaltohexaose (IM_6), 0.04 μmole of isomaltoheptaose (IM_7) and 0.15 μmole of methyl α-D-glucopyranoside (MG).

rides, and it can be seen that an increase in molecular weight results in a decrease of retention time on the column. Fig. 2 shows the separation of α-linked glucose disaccharides and their tetrahydroborate-reduced products. Kojibiose and maltose were separated from nigerose and isomaltose, but not from each other, nor were nigerose and isomaltose resolved from each other. Similarly, nigeritol and kojibiitol were separated from maltitol and isomaltitol, but not from each other, nor were isomaltitol and maltitol resolved from each other.

Application of the system to an acetolysate of dextran

Dextran NRRL B1355 S was acetolysed and de-acetylated. Two major peaks and at least five trace compounds were recorded when the sugars were chromato-

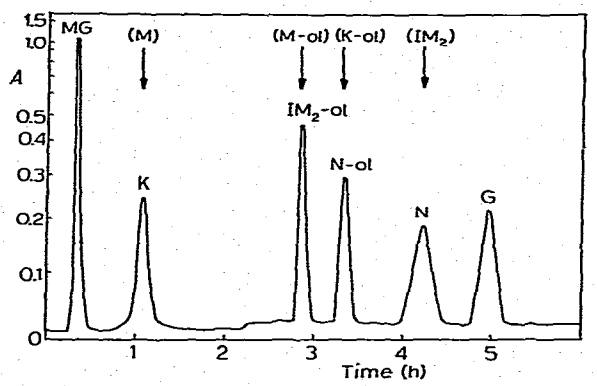

Fig. 2. Chromatographic separation, under conditions (i), of a mixture containing 0.2 μmole of D-glucose (G), 0.13 μmole of kojibiose (K), 0.13 μmole of nigerose (N), 0.2 μmole of nigeritol (N-ol), 0.2 μmole of isomaltitol (IM_2-ol) and 0.2 μmole of methyl α-D-glucopyranoside (MG). Locations of maltose (M), isomaltose (IM_2), kojibiitol (K-ol) and maltitol (M-ol) are shown in parentheses.

graphed. The largest peak corresponded to glucose, and the other major peak was in a position corresponding to that of nigerose and isomaltose. The sugars were reduced with sodium tetrahydroborate and again subjected to chromatography. Only one orcinol-positive substance (nigeritol) was detected.

Quantitation of the identified sugars by automated analysis showed that this dextran liberated 46.9% of glucose and 30.7% of nigerose on acetolysis, indicating that at least 30.7% of nigerose units were contained in its structure. Although Jeanes *et al.* had shown, by periodate oxidation[11], that dextran B1355 S contained 57% of (1→6), 35% of (1→3)-like and 8% of (1→4)-like linkages, neither kojibiose nor maltose was found in the acetolysate in our experiments. Low values (40 to 69%) for total sugar recovery after acetolysis of dextrans were also reported by Suzuki and Hehre[15].

In this work, we have shown that various α-linked glucose oligosaccharides could be separated and quantitated by an automated analysis system. The results suggest a possible application of the system to oligosaccharide analysis.

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