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Note

Separation of 2-keto-3-deoxyoctonic acid and its derivatives by ion-exchange chromatography

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In the course of studying the structure of bacterial lipopolysaccharides, it was necessary to separate acidic products containing 2-keto-3-deoxyoctonic acid (KDO), a common component of lipopolysaccharides, or KDO derivatives from neutral sugars, polyols and inorganic salts. These compounds resulted from mild hydrolysis or periodate oxidation of lipopolysaccharides. Two methods are currently used for the preparation of KDO fractions: gel permeation on Sephadex G-10 or G-15 for the separation of polysaccharide fragments from KDO after an acetic acid treatment of the lipopolysaccharide[1], and paper electrophoresis for the separation of acidic and neutral sugars after partial degradation of the lipopolysaccharide or polysaccharide[2–4]. We have found this latter method unsatisfactory when large quantities of inorganic salts accompanied acidic sugars. In this paper we report a rapid and efficient method which provides the complete separation of acidic sugars.

EXPERIMENTAL

Materials

Amberlite IR-45 and Amberlite IR-120 were obtained from Touzart et Matignon (Vitry/Seine, France).

Methods

The preparation and the purification of the lipopolysaccharide from *E. coli* K 12 CR 34 have been previously described[5]. The lipopolysaccharide was hydrolyzed by 1% acetic acid for 45 min at 100° and the water-soluble part was fractionated on a column of Sephadex G-15; the first eluate ($K_D = 0.20$) was the polysaccharide and the second eluate ($K_D = 0.80$) was the KDO fraction. A KDO derivative was obtained by degradation according to Goldstein *et al.*[6] of the dephosphorylated polysaccharide (unpublished results).

Amberlite IR-45 was gently stirred in 2 *N* formic acid for 20 min, filtered on sintered glass and washed with distilled water until it reached pH 3.5. Amberlite IR-120 was treated in the same way in 1 *N* HCl and washed until it reached pH 4.5. Ion-exchangers were used in the columns (15 × 1.5 cm I.D.).

KDO containing fractions (20 mg) were applied on the Amberlite IR-45

column and elution was performed by 100 ml water which eluted neutral products and then by 30 ml of 5% solution of sodium formate. The formate eluate was desalted on a column of Amberlite IR-120 with elution by 100 ml water. The eluate was evaporated *in vacuo* to dryness.

The analysis of KDO-containing compounds was performed by paper electrophoresis on Whatman 3 MM paper, in a pyridine–water–acetic acid (2.5:1000:9) buffer, pH 4, at 30 V/cm for 80 min. The spots were visualized by spraying the paper with an ammoniacal silver nitrate solution[7].

RESULTS AND DISCUSSION

The KDO fraction from an acetic acid hydrolysis of the lipopolysaccharide from *E. coli* K 12 CR 34 was analyzed by the above method. One silver nitrate positive spot was obtained by paper electrophoresis of the sodium formate eluate with the same electrophoretic mobility as the standard KDO.

The polysaccharide dephosphorylated by treatment with hydrofluoric acid[8] and degraded according to the method of Goldstein *et al.*[6], gave a complex mixture of glycerol, glyceraldehyde, ethyleneglycol and an oligosaccharide containing an acidic KDO derivative. A direct paper electrophoresis of this mixture showed no separation as the solution contained large amounts of inorganic salts. This solution was applied to the Amberlite IR-45 column, as described above, and a pure acidic oligosaccharide was obtained.

This ion-exchange fractionation is an improvement of previous methods as it allows a rapid separation of acidic sugars and acidic oligosaccharides from neutral compounds and inorganic salts. Formic acid, which was used as the eluting solvent, is rapidly eliminated by evaporation *in vacuo* with no hydrolysis of glycosidic linkages. The acidic compounds can be directly used for further structural determinations.

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