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Bacterial lipopolysaccharide as a void volume marker for agarose gel permeation chromatography

The current wide availability of agarose preparations for molecular exclusion chromatography has provided a convenient technique for the separation of high molecular weight macromolecules. The use of agarose preparations having exclusion limits above a molecular weight of 2×10^6 presents a problem in the determination of the void volume of the column, since there is no commercial marker available for such determinations.

The author has found that a preparation of lipopolysaccharide from Gram-negative bacteria serves as a convenient marker for the determination of the void volume of agarose columns with an exclusion limit of 1.5×10^8.

Materials and methods

Agarose gels. Columns were prepared using spherical beads of agarose obtained from Bio-Rad Laboratories (Richmond, Calif.) and from Pharmacia Fine Chemicals, Inc. (Piscataway, N.J.). The Bio-Rad preparations included Bio-Gel A5, A15, A50 and A150, having molecular weight exclusion limits of globular materials of 5×10^6, 15×10^6, 50×10^6 and 150×10^6, respectively. Sepharose 2B, having an exclusion limit of 25×10^6, was the only Pharmacia preparation tested. The columns used were 2.5 cm in diameter, ranged from 30–45 cm, in length, and were packed in accordance with the instructions of the manufacturer. Both ascending and descending elution was used at various times. After packing, the columns were washed with one to two bed volumes of 0.05 *M* Tris(hydroxymethyl)aminomethane buffer, pH 7.2.

Lipopolysaccharide. For most of the work reported here, phenol–water extracts of *Salmonella typhimurium* were prepared as described by LÜDERITZ, STAUB AND WESTPHAL[1]. A similar preparation of *S. typhosa* 0901, obtained from Difco Laboratories (Detroit, Mich.), was also utilized.

Methods

Samples of 2 to 10 mg in a volume of 0.5 to 2 ml (less than 1% of the bed volume) were applied to the column and eluted with Tris buffer as described above. Fractions of 5 ml were collected volumetrically and each fraction assayed for carbohydrate by the phenol–sulfuric acid technique of DUBOIS *et al.*[2]. Tubes containing known amounts of lipopolysaccharide were interspersed throughout the series of collected fractions during assay to verify the accuracy of the procedure.

Results and discussion

The recovery of the lipopolysaccharide prepared in this laboratory was quantitative, with all the material eluting essentially in one peak at (approximately 25% of the bed volume) the expected void volume of the column (Fig. 1). The Difco preparation was found to yield two minor peaks following the major peak closely. These materials should not reduce the efficacy of the preparation as a void volume marker. The structure of lipopolysaccharide as shown by SHANDS *et al.*[3] and RUDBACH *et al.*[4] is that of a fibrillar network yielding an extremely high molecular diameter. The

final step in the purification of the lipopolysaccharide, centrifugation at 105,400 × *g* for 1 h, is essential to ensure that the material is eluted as a single peak. Crude extracts yield two peaks on high exclusion limit agarose preparations such as A150.

Since the DuBois procedure is a general technique for the detection of carbohydrate, the column must be washed with a minimum of two bed volumes of eluant prior to the loading of the column. Assay of eluant from columns left standing for several days showed small amounts of carbohydrate in the eluant, indicating the leaching of carbohydrate from the gel.

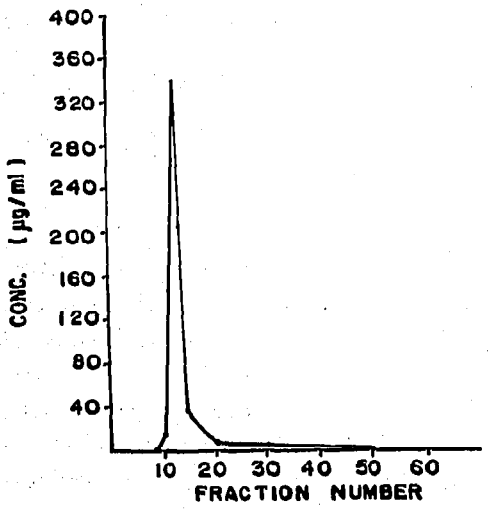

Fig. 1. A typical elution pattern of lipopolysaccharide from *S. typhimurium* on A150. The bed volume was 248 ml. A sample of 4 mg was applied to the column.

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