CHROM. 5227

Purification of *Pseudomonas aeruginosa* endotoxin by gel filtration on Sepharose 4B

Protein–lipopolysaccharide complex (Pr–LPS) from the cell walls of *Ps. aeruginosa* has been described by several authors[1,2]. The present study was undertaken to purify such a complex by means of gel filtration.

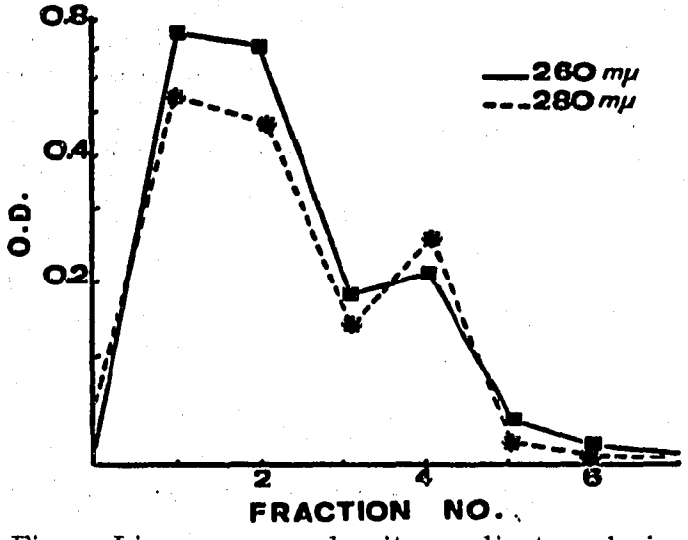
The cell walls were prepared from the pathogenic *Ps. aeruginosa* strain 115 according to the method of COX AND EAGON[1] without enzymatic treatment. Pr–LPS complex was obtained by incubation with EDTA as described by ROGERS *et al.*[2].

Proteins were estimated by the LOWRY's method[3] and total carbohydrates were determined by the anthrone method[4]. Isopycnic sucrose gradient centrifugation was carried out over 10 ml of a linear sucrose gradient (0.5–2 *M* sucrose); a 2 ml sample of Pr–LPS complex (1.5 mg/ml in 0.15 *M* NaCl) was centrifuged for 6 h at 0° and 35,000 r.p.m. on a Martin Christ Model Omega centrifuge. Fractions of 1 ml were collected with an Isco gradient fractionator by injecting a 3 *M* sucrose solution into the bottom of the centrifuge tube.

Gel filtration chromatography was performed on Sephadex G-200 and Sepharose 4B in columns (K 25/45 model from Pharmacia, Uppsala, Sweden). The columns were eluted with either 0.02 *M* Tris–HCl + 0.1 *M* NaCl (pH 7.6) or 0.02 *M* Tris–HCl + 1 *M* NaCl (pH 8) at room temperature.

Samples of 2 ml were collected on a Beckman fraction collector and registered in a Beckman DB-G spectrophotometer equipped with a continuous flow microcell with 0.1 ml in the light path.

Ultracentrifuge analyses were carried out in a Spinco model E ultracentrifuge. The observed sedimentation coefficients reported in Svedberg units were corrected to values corresponding to a solvent having the density and viscosity of water at 20°.



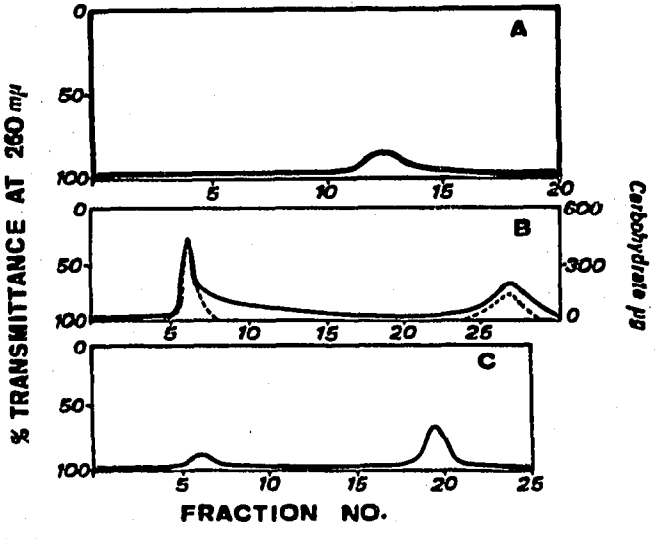


Fig. 1. Linear sucrose density gradient analysis of the Pr–LPS complex obtained from *Ps. aeruginosa*.

Fig. 2. Separation of the Pr–LPS complex from *Ps. aeruginosa* on: (A) Sephadex G-200; (B) Sepharose 4B eluted with 0.02 *M* Tris–HCl + 0.1 *M* NaCl (pH 7.6) buffer; (C) Sepharose 4B eluted with 0.02 *M* Tris–HCl + 1 *M* NaCl (pH 8) buffer. Transmittance (————); carbohydrate (– – – –).

Fig. 1 shows the two peaks obtained for the Pr–LPS complex when it was centrifuged on a linear sucrose gradient. It is clear that the components of the preparation could not be separated by this method. A single peak was obtained when the Pr–LPS complex was chromatographed on a Sephadex G-200 column as was shown previously[2], while two fractions were collected when the Pr–LPS complex was chromatographed on Sepharose 4B (Fig. 2); as shown, the two fractions obtained on Sepharose 4B were quantitatively different depending on the eluant used.

Tests for protein and carbohydrates were made on both fractions. The first peak was practically RNA-free with no absorption at 260 mμ while the second one contained some RNA impurities (13.2 % total weight) as determined by the absorption at 260 mμ.

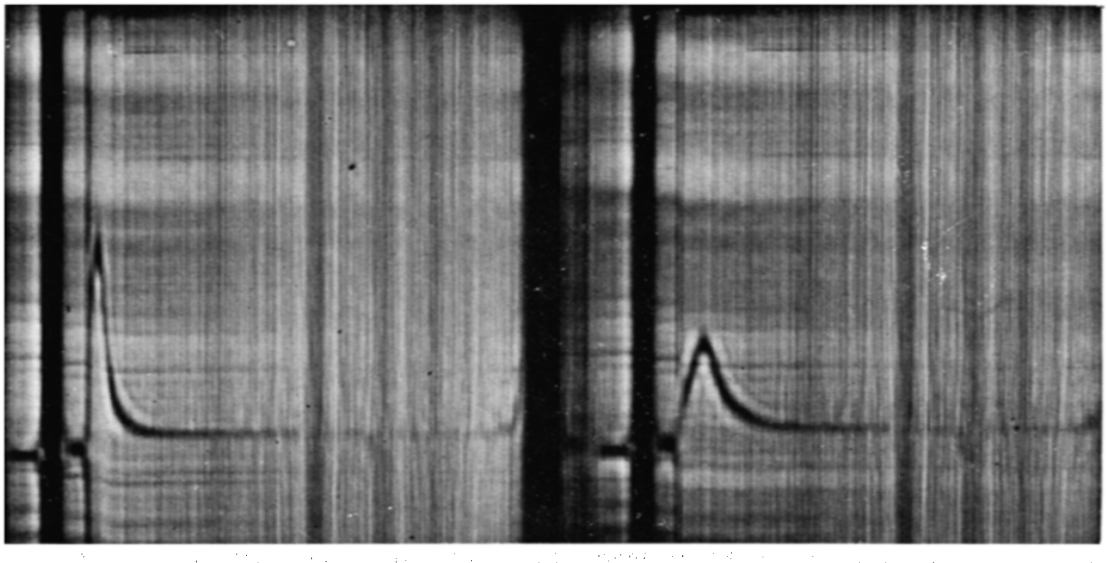


Fig. 3. Ultracentrifuge pattern of the first fraction obtained on Sepharose 4B chromatography. The photographs were taken at 1 min (left) and 34 min (right) after reaching a maximal speed of 20.410 r.p.m. The sample concentration was 5 mg/ml in 0.15 *M* NaCl.

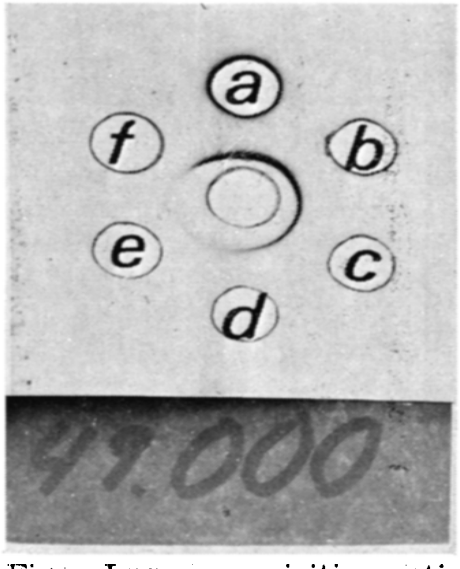


Fig. 4. Immunoprecipitin reaction of the first fraction obtained on Sepharose 4B (center well) and 1/2 (a) to 1/64 (f) anti Pr–LPS serum dilutions.

The purity of the first fraction obtained on Sepharose 4B was tested by ultracentrifugal analysis with the result that a single peak with an $S_{20,w}$ of 13.4 was obtained (Fig. 3). In addition, an immunodiffusion test revealed a single line against different dilutions of the antiserum to the Pr–LPS complex (Fig. 4).

Our results suggest that the Sepharose gel filtration is a good procedure for obtaining pure Pr–LPS fraction in a short time, and that it can be used for immunological purposes.

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Excessive baseline drifts during liquid ion-exchange chromatography of amino acids

Baseline stability and reagent purity were negligible problems during amino acid analysis by automated two-column ion-exchange chromatography of complex mixtures at micromolar levels. Unacceptable irregularities in the chromatogram emerged when the sensitivity was increased to nanomolar levels and when one-column rather than two-column assay was employed. Symptoms of the problems were (1) an abrupt rise and fall in background to form an elevated plateau overrunning several compounds that were eluted in the region of ammonia, and (2) an inconsistent, gradual rise or fall of the baseline at various other regions on the chromatogram.

As many colleagues have suspected, the primary cause of the first symptom appeared to be ammonia contamination in the buffers, despite extensive precautions to the contrary. Tedious, systematic attempts to determine the origin of the contaminant(s) included trials of new lots of buffer salts, greater care in cleaning and handling containers, triple redistillation of acidified tap and well water from various sources, filtration of the redistilled water through ion-exchange and charcoal columns, redistillation of reagent grade HCl from various sources, tests of antioxidants (thiodiglycol), substitution of preservatives (octanoic acid, pentachlorophenol), and elimination of wetting agent (BRIJ-35). All of these gave marginal improvement only. The citrate and chloride salts of lithium, although of reagent grade purity, emerged as prime suspects. Tobacco smoke and transient vapors from other laboratories beyond