Isolation of human apolipoprotein E by high performance liquid chromatography

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We were very interested in the method reported by Pfaffinger, Edelstein, and Scanu (1) for a rapid isolation of apolipoprotein E using molecular sieve high performance liquid chromatography. We were, however, puzzled by the recommended column configuration and did not understand the rationale for using, in series, a Bio-Gel TSK 50, a Bio-Sil TSK 400, and a Spherogel TSK 3000 column. The selection of one TSK 3000 column seemed sufficient to separate apoE (mol wt 35,000) from the apoC peptides (mol wt 7,000–13,000) and from apoB (mol wt > 200,000). As the use of a single column substantially decreases the cost of the procedure and speeds up the separation, we report our experience using one TSK 3000 column for apoE fractionation.

The apoVLDL sample was prepared by VLDL delipidation with ether–ethanol 3:1 (v/v) and solubilization in 6 M GdmCl solution, 0.1 M Tris, 0.01 M dithiothreitol, pH 7.0, for 12 hr (2). The sample was either filtered through a 0.2-μm Millipore filter or spun at 100,000 *g* in an Airfuge (Beckman Instruments, Palo Alto, CA) for 10 min. We used an Ultro-Pac TSK-G 3000 SW, 600 × 7.5 mm column (LKB, Bromma, Sweden) and an Ultro-Pac TSK-GSWP guard column 75 × 7.5 mm (LKB). The column system was eluted with a 4 M GdmCl solution in 0.1 M Tris, 0.01 M dithiothreitol, pH 7.0. The separation was carried out on an LKB 2150 liquid chromatograph at flow rates of 0.25 and 0.5 ml/min at 23°C (3). A 200-μl sample containing 2 mg of apoVLDL was injected. The effluent was monitored at 280 nm and fractions of 0.5 ml/tube were collected (3), dialyzed against $5 \cdot 10^{-3}$ M NH_4HCO_3, lyophilized, and stored at −20°C.

A base-line resolution was obtained for three major peaks eluting at 63.8, 82.5, and 106 min, and a minor component at 94.5 min. The percent areas of the major peaks agree with the previous report (1). Only traces of the apoE-A-II complex reported by Pfaffinger (1) were apparent, as the sample was solubilized in the buffer for 12 hr. The lyophilized fractions were further identified by polyacrylamide gel electrophoresis on 10% gels in the presence of 0.1% SDS, after reduction with β-mercaptoethanol. The three major peaks, identified by comparison with an apoVLDL pattern and by molecular weight estimation, correspond to apoB, apoE, and apoC, respectively. The purity of the apoE fraction was further tested by immunodiffusion using rabbit monospecific antisera against apoA-I, A-II, B, C-II, C-III, and E. We observed a positive reaction against anti-apoE only. The same degree of purity was obtained for the apoE fraction when the column was eluted at a flow rate of 0.5 ml/min. The column recovery for apoVLDL was 95%, as calculated from mass determination of the applied sample and from the sum of the eluted fractions.

In conclusion, we feel that the use of the single TSK 3000 column, instead of the column system used by Pfaffinger et al. (1), is sufficient for the isolation of pure apoE. This represents a substantial saving in the purchase of these high cost columns and should promote the application of the HPLC technique for apoprotein fractionation.

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