notes on methodology

Preparative electrophoresis of human apolipoprotein E: an improved method

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Summary Apolipoprotein E was isolated from human very low density lipoproteins by a two-step electrophoretic procedure derived from that of Méndez (1982. *Anal. Biochem.* **126:** 403–408). It included separation in a sodium dodecylsulfate polyacrylamide slab gel, transfer into an agarose gel, and extraction by ultracentrifugation for 30 min. No protein labeling, dialysis, or concentration procedures were needed. The method was fast, showed an excellent protein recovery, and could be suitable as a general method of protein isolation by polyacrylamide gel electrophoresis.—**Meunier, S., P. Gambert, J. Desgres, and C. Lallemant.** Preparative electrophoresis of human apolipoprotein E: an improved method. *J. Lipid Res.* 1986. **27:** 1324–1327.

Supplementary key words very low density lipoproteins • isoelectric focusing • apoE isoforms • electrophoretic elution

The high resolution of electrophoresis in polyacrylamide gels should make it a method of choice for the isolation of proteins. However, its use as a preparative procedure is impeded by the generally complicated, lengthy, and nonquantitative extraction from the gel. Problems of extraction are increased when proteins have a tendency to interact with surfaces and to aggregate, as is the case for apoE (1). In spite of that, several authors have used electrophoretic procedures for the isolation of apoE (2–4). Protein extraction required crushing of the gel (4), continuous elution (2), or electrophoretic elution into dialysis sacks (3). We have found that the procedure could be greatly improved by using a method, derived from that of Méndez (5), which is characterized by an electrophoretic transfer of apoE into an agarose gel and extraction of the protein from this gel by a short ultracentrifugation.

MATERIALS AND METHODS

Chemicals

Urea and SDS were from Prolabo (Paris, France); acrylamide was purchased from BDH Chemicals (Poole, England); Coomassie brilliant Blue G 250 and dithiothreitol were obtained from Sigma (St Louis, MO). Agarose IEF, ampholyte, and a low molecular weight protein calibration kit were from Pharmacia (Uppsala, Sweden). All other chemicals were from Merck (Darmstadt, F.R.G.).

Polyclonal antibodies were from Immuno (Vienna, Austria). Monoclonal antibodies against human apoE (6) were a generous gift from Dr. Y. Marcel and Dr. R. W. Milne (Institut de Recherches Cliniques de Montréal, Canada).

VLDL apolipoprotein preparation

Four ml of serum from normolipidemic or hypertriglyceridemic subjects was overlayered with 4 ml of 0.15 M NaCl in cellulose nitrate tubes. The tubes were placed in a Beckman 65 rotor and centrifuged for 22 hr at 105,000 *g* in an L2 65B ultracentrifuge (Beckman, Palo Alto, CA). VLDL were recovered in the top 0.6 ml.

The VLDL preparation was delipidated at room temperature with two volumes of butanol-diisopropyl ether 40:60 (v/v) according to the procedure of Cham and Knowles (7). The tubes were shaken for 3 min on a vortex mixer, for 1 hr on a rotating mixer (22 times/min), and for 3 min on a vortex mixer. The infranate was collected after a 3-min centrifugation at 10,000 *g*. This delipidation step was omitted when the triacylglycerol concentration of the VLDL preparation was below 50 g/l. At that low concentration, the protein recovery was better and the electrophoretic separation was not impaired.

Delipidated or not, the VLDL preparation was mixed with 0.5 vol of a reducing solution of 100 g/l SDS, 66 g/l dithiothreitol, 0.24 mol/l Tris–HCl (pH 6.7) and incubated for 5 min at 100°C.

ApoE preparation

The separation of VLDL apolipoproteins was carried out, according to the general method of Davis (8), in a vertical 10 × 22 × 0.2 cm acrylamide slab gel. A 140 g/l acrylamide separation gel was placed under a 1-cm layer of a 34 g/l acrylamide concentration gel. The concentration of Tris–HCl buffer in the upper gel was 0.06 M (pH 6.7) and 0.5 M (pH 9.1) in the lower gel. In both gels the concentration of diallyltartardiamide, the cross-linking agent, was 10% (w/w) of the acrylamide concentration and both gels contained 1 g/l SDS. The running buffer was 49 mM Tris–380 mM glycine (pH 8.3) with 1 g/l SDS. The VLDL apolipoprotein preparation (2–8 mg

Abbreviations: apoE, apolipoprotein E; VLDL, very low density lipoproteins; SDS, sodium dodecylsulfate.

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of protein in about 2 ml) was layered on the concentration gel in a 13-cm large central slot. Two 5-μl aliquots from the protein calibration kit were placed in 0.5-cm lateral slots. The electrophoresis was performed at 100 V with a 40 mA current for the first hour and at 200 V with an 80 mA current for the following 4 hours.

After guiding lines were incised on the surface of the gel, apoE was located by cutting off and staining (9) the lateral portions of the gel containing the calibration kit and the edge of the VLDL apolipoprotein separation. The apoE band was removed from the remaining unstained gel and placed on the top of a vertical 7 × 22 × 0.2 cm agarose IEF slab gel held by a bottom layer of a 50 g/l polyacrylamide gel. The protein was transferred to the agarose gel by a 2-hr electrophoresis at 70 mA. Agarose concentration was 8 g/l in 0.24 M Tris–HCl buffer (pH 6.7). The migration buffer was 49 mM Tris–380 mM glycine (pH 8.3). The gel and the migration buffer contained 1 g/l SDS.

ApoE was seen in the agarose gel as a refringent band that was sliced out and placed in a polycarbonate ultracentrifuge tube. After centrifugation in a 65 rotor of an L2 65B centrifuge for 30 min at 105,000 *g*, the protein (100–500 μg) was recovered in about 1.5 ml of supernatant.

When necessary, i.e., for amino acid analysis and isoelectric focusing, the apoE solution was lyophilized and successively extracted with trichloracetic acid solution (200 g/l) and acetone according to the procedure described by Kane, Hardman, and Paulus (10) in order to remove SDS and salts.

Analytical methods

Polyacrylamide electrophoresis. Disc electrophoresis in 140 g/l acrylamide gel was conducted in 12.5 × 0.5 cm glass tubes according to the procedure described above for the preparation of apoE by preparative slab gel electrophoresis. Gradient gel electrophoresis was performed as previously described (11). The linear gradient of polyacrylamide ranged from 25 to 300 g/l. All buffers contained 2 g/l SDS.

Isoelectric focusing was conducted according to Warnick et al. (12) in a 75 g/l acrylamide gel containing 50 ml/l of ampholyte (pH 4–6.5) solution and 8 M urea.

ApoE was assayed by a competitive enzyme immunoassay using a monoclonal anti-apoE antibody (unpublished procedure).

Amino acid assay. After hydrolysis of apoE by 6 M HCl at 110°C for 20 hr and 40 hr under nitrogen, amino acids were separated and quantified by gas–liquid chromatography on a glass capillary column (13).

RESULTS AND DISCUSSION

In preparative polyacrylamide gels, VLDL apolipoprotein bands appeared linear and well separated (**Fig. 1**).

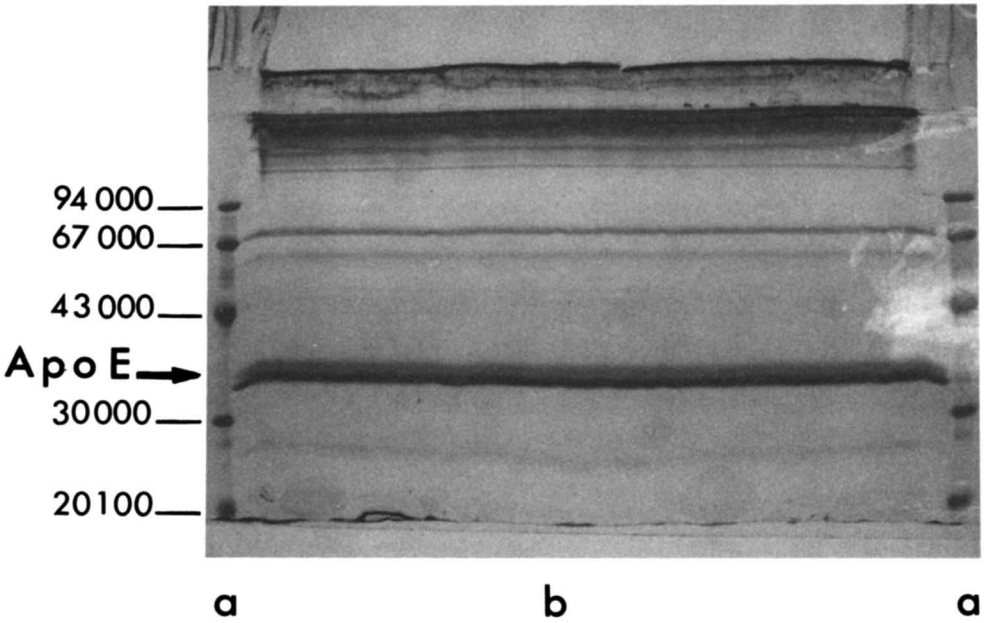

Fig. 1. Preparative polyacrylamide gel electrophoresis of VLDL apolipoproteins. a), Low molecular weight calibration kit (94,000, phosphorylase b; 67,000, albumin; 43,000, ovalbumin; 30,000, carbonic anhydrase; 20,100, trypsin inhibitor). b), VLDL apolipoprotein preparation (VLDL were prepared from 10 ml of a pool of moderately hypertriglyceridemic sera).

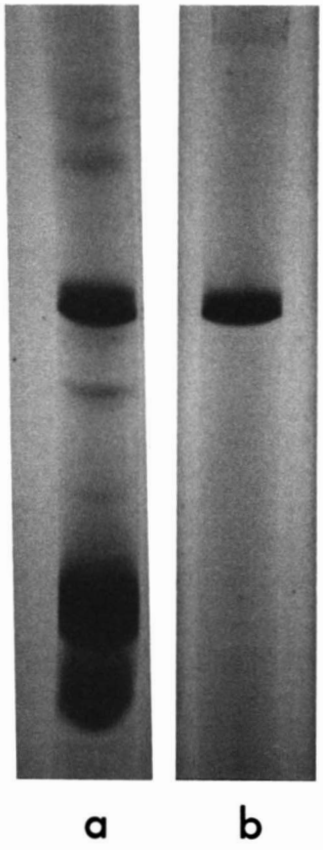

Fig. 2. Analytical polyacrylamide gel electrophoresis of VLDL apolipoproteins (a) and isolated apoE (b). Amounts of apoE were 11 μg in (a) and 7.7 μg in (b).

Edge effects were limited and did not impair the resolution of the separation. ApoE was the only protein in the 30,000–43,000 molecular weight range which stretched over a 2.5-cm long portion of the gel. Thus, there was no risk of contamination by other apolipoproteins. In VLDL apolipoprotein preparations from pools of sera, a diffuse slower band was generally seen close to the main apoE band (Fig. 1). Such a band has been already described by other authors and attributed to sialylated derivatives of apoE (14). This accessory band was usually collected with the main band and was detected in the apoE preparation by analytical disc electrophoresis (**Fig. 2**) but not by gradient gel electrophoresis (not shown). In order to ascertain the apoE nature of the accessory band, the two bands were separately collected. Isoelectric focusing of the two preparations showed that both possessed a typical apoE pattern (**Fig. 3**). However, compared with the main band composition there was, in the accessory band, a relative increase of the cathodic apoE isoforms and a decrease of the anodic isoforms, a difference of composition that explains the difference in migration rates. Thus, high resolution polyacrylamide electrophoresis can partially separate apoE isoforms, as previously suggested by Warnick et al. (12), and can lead to preparations free of non-apoE proteins.

The isolated protein was identified as apoE by its molecular weight, its amino acid composition, and its immunological properties. By comparison with that of a protein calibration kit, the migration of the protein in SDS polyacrylamide gels indicated an apparent molecular weight of 35,000. This value is in good agreement with the value (34,145) calculated from the amino acid sequence of the apoE2 isoform (15). The amino acid composition (**Table 1**) showed the arginine-rich feature of the protein. It was comparable to compositions previously published. Whereas immunodiffusion tests carried out with specific antisera against apolipoproteins A-I, A-II, and B were negative, positive reactions were observed with anti-apoE monoclonal antibodies.

Electrophoretic elution from a polyacrylamide gel into an agarose gel, followed by ultracentrifugal extraction was well suited to the preparation of apoE. Changes in

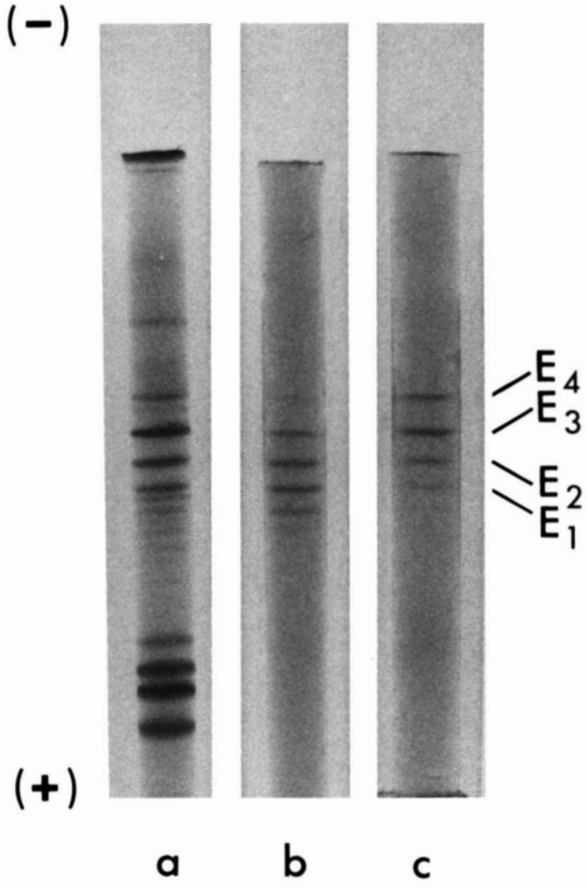

Fig. 3. Isoelectric focusing patterns of a) a VLDL apolipoprotein preparation; b) the main apoE band; and c) the accessory slow apoE band. The nomenclature of apoE isoforms is that of Warnick et al. (12).

TABLE 1. Amino acid composition of isolated apoE

Amino Acid[a]	mol/100 mol[b]
Alanine	12.6
Arginine	12.3
Aspartic acid + Asparagine	4.0
Glutamic acid + Glutamine	22.8
Glycine	5.6
Isoleucine	0.8
Leucine	15.3
Lysine	5.1
Methionine	0.9
Phenylalanine	1.5
Proline	3.2
Serine	4.4
Threonine	3.0
Tyrosine	1.4
Valine	7.1

ApoE was isolated from the VLDL fraction of a pool of hypertriglyceridemic sera.

[a] Cysteine, histidine, and tryptophan were not determined.

[b] Results expressed per 100 mol of determined amino acid.

the technique of Méndez (5) improved the practicability and the efficiency of the method. Protein labeling was eliminated. ApoE was prepared from small volumes of serum and the recovery was high. In typical experiments, about 400 μg of apoE was prepared from 10 ml of a pool of moderately hypertriglyceridemic (4–5 g/l) sera. ApoE enzyme immunoassay in VLDL apolipoprotein preparations and in an apoE solution showed a recovery of approximately 90%. Addition of a concentration step to the electrophoretic process led to focused apoE bands in the polyacrylamide and agarose gels. Consequently apoE was extracted from a small volume of agarose gel and the solution recovered by ultracentrifugation was concentrated enough (about 250 μg/ml) to dispense with further concentrating procedures.

Beyond its application to apoE, the present method is rapid, needs no special apparatus, can be adapted to various amounts of protein, and results in an excellent recovery of protein. It could be suitable as a general method of protein isolation by polyacrylamide gel electrophoresis.

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