papers and notes on methodology

Analytical isoelectric focusing with immobilized pH gradients of human apolipoprotein E from very low density lipoproteins and total plasma

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Abstract A method for analytical isoelectric focusing (IEF) of apolipoprotein E (apoE) in immobilized pH gradients (IPG) and immunodetection of the separated isoforms has been developed for use with either very low density lipoproteins (VLDL) or whole plasma. Both VLDL and plasma were sequentially delipidated with 1,4-dioxane, acetone–ethanol, and ether. Neuraminidase treatment preceded the delipidation when required. Using preformed plates, pH 5.0–6.0 (LKB, Bromma) after rehydration with 6 M urea and dextran T-10, the IPG focusing pattern of the common isoforms (E2, E3, E4) was found to be equivalent to conventional IEF with the added resolution of the E4 disialo form. The use of self-poured narrower gradients permitted the further resolution of the E4 monosialo form, a previously unrecognized heterogeneity of the E2, E3, and E4 monosialo isoforms and differentiation of the apoE2** mutant; all of these forms comigrate with the common isoproteins in conventional IEF. Finally, the conditions for IPG of whole plasma using apoE monoclonal antibodies and enzyme-conjugated anti-mouse IgG for detection were established. Thus, IPG focusing is shown to be a powerful method for resolution of the apoE sialoforms and apoE mutant forms. The method has important implications in accurate and diagnostic phenotyping. Moreover, it is a convenient method for phenotyping which requires only very small volumes of plasma. — **Mailly, F., J. Davignon, and A. C. Nestruck.** Analytical isoelectric focusing with immobilized pH gradients of human apolipoprotein E from very low density lipoproteins and total plasma. *J. Lipid Res.* 1990. **31:** 149–155.

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In man, apolipoprotein E (apoE) is a polymorphic protein (1) which is found on the surface of several classes of the plasma lipoproteins. One of its major metabolic roles is to act as a ligand for the hepatic and peripheral receptor-mediated clearance of these lipoproteins (2). Three codominant alleles at a single locus on chromosome 19 give rise to three major plasma forms which determine six common apoE phenotypes. The isoforms, designated E2, E3, and E4, differ by single, amino acid substitutions (cysteine for arginine) at two sites, residues 112 and 158, of the 299 amino acid polypeptide chain (3). As separated by isoelectricfocusing (IEF), apoE4 is the most basic form with arginine at both variant sites; apoE3 has intermediate migration with cysteine at site 112 and arginine at 158; and apoE2 is the most acidic form with cysteine at both sites. Additionally, the post-translational addition of sialic acid residues to the polypeptide chain, followed by partial desialylation in the plasma results in the production of mono (-1S) and disialylated (-2S) derivatives for each of the isoforms (4). This determines a second level of variation superimposed on that of genetic orgin. Further, several mutant apoE proteins, many of which comigrate with the major isoforms on IEF, have also been characterized (5–11).

It is now well known that genetic variation at the apoE locus affects both the binding affinity and degradation kinetics of the apoE isoproteins, contributes significantly to variation in plasma levels of cholesterol and triglycerides, and predisposes to development of some types of dyslipoproteinemia (for a recent review see ref. 12). For these reasons, accurate determination of the apoE phenotype and detection of rare genetic variants at this locus are important.

The earlier methods for apoE phenotyping using IEF in carrier ampholyte-generated gradients were complicated and often required second-dimension electrophoresis and treatment with cysteamine for resolution. A one-dimensional method, developed by Bouthillier, Sing, and Davignon (13), simplified phenotyping and used VLDL isolated by ultracentrifugation as the source of apoE with direct staining of the cylindrical gels with Coomassie blue. Ordovas et al. (14) developed a minigel methodology with direct staining. More recently, several investigators have determined apoE phenotypes directly

Abbreviations: apo, apolipoprotein; VLDL, very low density lipoproteins; IEF, isoelectric focusing; IPG, immobilized pH gradient; TBS, Tris-buffered saline.

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from serum or plasma by immunological identification of the isoforms (15–17). Although these more recent methods eliminate costly and time-consuming ultracentrifugation, they are nevertheless faced with the inherent limitations of conventional carrier ampholyte gradients: cathodic drift of the gradient which limits both focusing time and narrowing of the gradient for increased resolution, and sensitivity to salt load. Thus, due to limited resolving power, all the conventional IEF methods result in the superimposition of the sialylated derivatives (one negative charge per sialic acid) on the major isoforms one charge more negative (i.e., E4-1S on E3, E3-1S on E2, etc.) which can confound phenotyping of heterozygous subjects. As the resolving power of the system can hardly be increased, one must either pretreat samples with neuraminidase or establish cut-off points from densitometric ratios of band intensity. Second-dimension analysis, with separation of proteins according to size, may be carried out to successfully resolve these forms (4). However, this technique is not well suited for the cost-effective screening of a large number of samples. As well, rare variant E2 and E3 forms superimpose on the common isoforms and can only be resolved through laborious procedures, such as binding assays and protein sequencing (6, 9, 10).

A new concept in IEF methodology, was introduced by Gasparic, Bjellqvist and Rosengren (18) and developed by Bjellqvist et al. (19), where the charged moieties that generate the pH gradient are fixed by co-polymerization to the polyacrylamide gel matrix. This prevents cathodic drift and makes ultra-narrow, stable gradients possible. Moreover, the system is extremely flexible in terms of the available gradient ranges, owing to the monomeric composition of the gradient-generating buffers, which allows formation of tailored gradients to fit specific needs. Immobilized pH gradients (IPG) have only recently been commercially available and have been successfully as an analytical method to separate mouse hemoglobins (20), human transthyretin forms differing only by neutral amino acid substitutions (21), as well as closely related human globin chains (22, 23) and alpha-antitrypsin isoenzymes (24). All were previously unresolved by conventional IEF. Additionally, Menzel et al. (25) have used IPG for the preparative focusing of apoA-I and Weisgraber et al. (26) used IPG for apoE and their respective minor isoforms. In addition to excellelnt resolution of closely related forms, this system allows the elution of the separated proteins without contamination by carrier ampholytes.

Based on this knowledge, we hypothesized that IEF in IPGs could be applied to the separation of the apoE sialo and asialo forms in a single dimension and to the detection of mutant forms, even those with neutral amino acid substitutions. We therefore decided to set up an analytical method for the high resolution of apoE isoforms using both VLDL and total plasma as samples.

MATERIALS AND METHODS

Preparation of samples

An apoE-enriched fraction from either plasma (1.5 mg/ml EDTA) or VLDL, isolated as descrided previously (13), was obtained by selective extraction with 1,4-dioxane, using a slight modification of the method of Holmquist and Carlson (27).

Delipidation of plasma was performed as follows. Twenty μl of plasma was diluted 1:1 with saline and VLDL apolipoproteins were solubilized by adding 80 μl of 1,4-dioxane, vortexing 10 sec, and centrifuging for 30,000 *g*-min. The liquid phase was carefully removed and the proteins were precipitated in 3–4 ml of acetone–ethanol 1:1 at $-20°C$ for at least 2.5 h. The proteins were spun at 2500 rpm for 10 min and the pellet was washed once with ethyl ether for 1 h. After removal of the solvent, the pellet was resuspended for 20 min in 45 μl of 0.01 M Tris-HCl, pH 10, 50 mM dithiothreitol (DTT), and 6 or 8 M urea, depending on the focusing gel used (Bio-Rad, electrophoresis purity reagents), and Ampholines pH 5–7 (LKB, Bromma). The final concentration of the Ampholines varied with the pH range of the gradient. For VLDL, the delipidation method was the same except for the following details. A volume of VLDL containing 50–75 μg of protein (13) was mixed undiluted with 2 volumes of dioxane, processed as for plasma, and resuspended in 20 μl of the same sample buffer as above.

When applicable, the samples were treated with neuraminidase (*Clostridium perfringens*, Type V (Sigma, St. Louis, MO)) in the following manner. An appropriate volume of either VLDL or plasma was diluted to 250 μl with physiological saline and mixed with an equal amount of reaction buffer (0.1 M CH_3COONa, 1.8% NaCl, 0.02% EDTA, 0.02% NaN_3, pH 5) containing 0.1 U of neuraminidase. Cysteamine modification of apoE isoforms was carried out according to Weisgraber et al. (3).

Preparation of the gels

Commercially prepared gels of pH range 5.0–6.0 (Immobiline Dry Plates, LKB Bromma) were used following rehydration with a solution of 6 M urea, 5% Dextran T10, 0.8% Ampholines pH 5–7 (LKB). The rehydration was continued until the gel absorbed 12.5 ml; 13.6 g by weight of 6 M urea.

We also poured our own gels on GelBond PAG films, using the LKB Multiphor II, 2117 gradient mixer, in a 125 × 260 mm mold with a 0.5-mm rubber gasket. The composition of the Immobiline monomer solutions (LKB, Bromma) was determined according to LKB Application note #324 (28) for gradients wider than 0.4 pH units, interpolating the required volumes of Immobiline monomer

solutions from the tables. For narrower gradients, we used the nomograms of LKB application note #321 (29), with a single buffering Immobiline monomer and a single titrant monomer. The solutions were 4% acrylamide (4% T, 3% C). Prior to pouring, the pH of the solutions was verified and corrected if needed with the appropriate titrant monomer, and subsequently adjusted to pH 7 with NaOH to insure even polymerization. The solutions, mold, and gradient mixer were cooled to 4°C prior to pouring as high levels of catalysts were used: 5 μl N,N,N¹,N¹-tetramethylethylenediamine and 7.5 μl 40% ammonium persulfate per chamber of the gradient mixer (each, 7.5 ml).

Polymerization time was 75 min at 50°C. The gel was then removed gently from the mold, washed in 500 ml distilled water for 20 min twice, and once in 500 ml 1% glycerol. The gel was dried at 40°C until thoroughly dry and rehydrated as above with 8 M urea (14 g by weight) containing Ampholines pH 5–7. The amount of Ampholines added to the rehydration solution depended on the range of the gradients: 0.8% for >0.7 pH units, 1.5% for 0.4–0.7 pH units, 2.0% for <pH units.

Electrophoresis

The rehydrated gel plate was put on the cooling plate of the electrophoretic apparatus, with the temperature pre-set at 15°C. Samples (20 μl) were applied near the anode on the surface of the gel, in a rectangle using the grid of the cooling plate as a guide. Focusing was started at 1.5 mA, with voltage limited to 5000 V and power to 5 W (Multiphor II, 2117 electrophoresis apparatus, a model 2297 Macrodrive 5 power supply (LKB, Bromma)). After 16 h, focusing was stopped, and excess liquid was wiped from the surface of the gel near the electrodes. For narrow gradients (<0.7 pH unit), focusing was continued for 2 h. For gradients of less than 0.2 pH units, we found it necessary to apply a higher starting current (4–5 mA).

Detection

Focused VLDL samples were visualized after direct staining. Gels were fixed in 400 ml, 11.5% trichloroacetic acid–3.5% sulfosalicyclic acid for 45–60 min. After 5 min equilibration in ethanol–acetic acid–water 25:5:70 (v/v), the gels were stained with 0.11% Coomassie blue R-250 in equilibration solution, which had been pre-heated to 60°C and filtered. The staining time was 10 min and destaining was continued until a clear background obtained.

For plasma samples a passive transfer to nitrocellulose (Hybond E, Amersham) followed by an immunoreaction (30) was used for detection. The focused gel was covered with the membrane wetted with Tris-buffered saline, pH 7.4 (TBS), two sheets of wetted (TBS) filter paper and several dry sheets, and sandwiched between two glass plates. The whole mount was then flipped upside down, several glass plates were added, and the transfer was allowed to proceed for 45 min. The nitrocellulose membrane was carefuly detached from the gel in the presence of TBS. Unoccupied binding sites were blocked by incubating with TBS, 4% BSA (Fraction V, Sigma) for 45 min at 37°C. The membrane was immunoreacted with mouse anti-apoE monoclonal antibodies (31) in TBS, 3% BSA for 2 h, followed by three 20-min washes with TBS, 1% BSA and a final incubation for 1 h with TBS, 3% BSA containing rabbit anti-mouse antibodies conjugated with peroxidase (32). The blocking, washing steps, and incubations with antibodies were carried out at 37°C. After additional washes with TBS, 1% BSA (2 × 30 min), the membrane was incubated with freshly prepared substrate solution (30 mg 4-chloro-1-naphthol in 8 ml 99.8% methanol, mixed with 40 ml TBS, to which 60 μl 30% H_2O_2 was added) until the bands were visible, rinsed with distilled water, dried, and stored in the dark.

RESULTS

The sequential extraction and delipidation of VLDL apolipoproteins was performed with 1,4-dioxane followed by acetone–ethanol and ether. This two-step procedure was applied to VLDL and total plasma.

The first experiment was designed to test the ability of IPGs to resolve the major apoE isoforms. Focusing was performed with VLDL samples from subjects with known apoE phenotypes using performed gradient gels (LKB, pH 5.0–6.0) in the presence of urea and carrier ampholytes. The resulting band patterns agreed with those obtained by conventional IEF (13) for the six phenotypes (**Fig. 1**). From their position in the focused gel, the apparent pI values of the asialo forms were estimated at: E4, 5.79; E3, 5.66; and E2, 5.55.

Additionally, when 5% Dextran T-10 was added to the rehydration solution, sharper bands were found. It was also possible to separate the E4 disialo from the E2 asialo form (Fig. 1). Neuraminidase treatment of the VLDL samples prior to delipidation caused the disappearance of sialo forms, as in conventional IEF. Treatment of samples with cysteamine gave the expected results: E2 isoforms moved towards the cathode by two charges, whereas E3 isoforms moved by one (results not shown).

Having estimated the apparent pI of the major isoforms in the commercially available gels, we set out to take advantage of the flexibility and high resolution of IPG by pouring the ultranarrow gradients best suited to our needs.

To further resolve the sialated and asialated species of apoE that comigrate (having the same total electric charge but slightly different structures), we focused apoE in a pH 5.3–5.9 gradient, containing urea and 1.5% carrier ampholytes. With these conditions, it was possible to sepa-

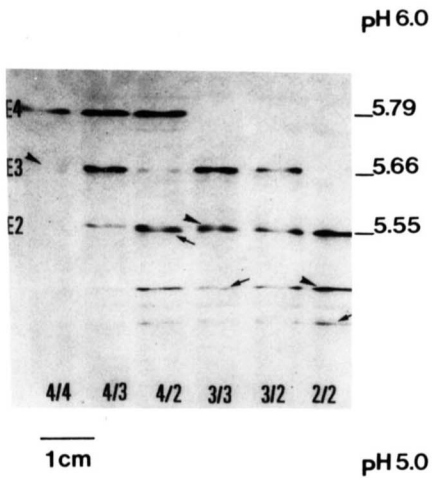

Fig. 1. The six apolipoprotein E phenotypes as determined by Immobiline isoelectric focusing of delipidated VLDL in a commercial dried gel (LKB, Bromma, pH 5.0–6.0), followed by protein staining (Coomassie blue R250). Arrowheads to the left of sample lanes indicate the position of monosialated (1S) isoforms; arrows to the right of sample lanes show disialated isoforms (2S). The estimated pIs of asialated forms are indicated at the right of the gel. The gel was rehydrated with a solution of 6 M urea, 0.8% Ampholines pH 5–7 (LKB), 5% Dextran T-10.

rate the E4-monosialo and the E3-asialo forms. All phenotypes were easily recognized in this gradient. Comparison with a conventional carrier ampholyte gradient of pH 4.0–6.0 shows the expansion of the gradient (**Fig. 2**).

Since we had not yet succeeded in resolving the E3-monosialo and E2-asialo forms, we made an even narrower gradient (pH 5.2–5.6), designed to examine specifically these isoproteins. Using this pH range, we uncovered the existence of heterogeneity in the E3 monosialo form focusing zone (**Fig. 3A**). Similar heterogeneity was also observed for the E2- (Fig. 3B) and E4-monosialo forms (result not shown).

We performed another experiment to show that this heterogeneity could be attributed to apoE. We focused delipidated plasma and VLDL with and without neuraminidase treatment. The results confirmed the presence of multiple sialo forms in VLDL or plasma, with the upper sialoform band (highest pI) showing resistance to the sialidase activity and comigration with the E2-asialo form (Fig. 3C). This heterogeneity could not be observed for the disialo forms (Fig. 3C).

The ability of IPGs to distinguish apoE isoproteins with minor amino acid sequence changes was also assessed in a pH 5.2–5.6 gradient, using a mutant apoE form for which the sequence defect is known (apoE2**, $Lys_{146} \rightarrow Gln$). ApoE2** was seen to focus slightly closer to the cathode than the common E2 isoform ($Arg_{158} \rightarrow Cys$) (**Fig. 4**).

Finally, we developed the conditions for apoE phenotyping with IPGs from total plasma. Twenty μl of plasma was usually delipidated and resuspended prior to focusing in 40 μl of sample buffer containing 6 or 8 M urea. Following the transfer to nitrocellulose, reaction with monoclonal antibodies, and immunodetection by enzyme reaction, the focused proteins produced regular band patterns, which agreed with those obtained using isolated VLDL as samples. We treated samples with neuraminidase to help distinguish between E3/3 and E3/2 phenotypes. Background was minimal providing that the washing step following the incubation with the monoclonal antibody was performed long enough (1.5 h with two or three changes of buffer) (**Fig. 5**).

DISCUSSION

We have developed a method for the improved study of apoE isoforms in immobilized pH gradients using several pH ranges. The Immobiline system has several advantages when compared to carrier ampholyte IEF: complete stability, reduced sensitivity to salt load, flexibility in the choice of the gradient, and uniform buffering capacity throughout the gradient (28). The IPG gels gave phenotype patterns in agreement with results previously obtained by conventional IEF. In addition, they allowed direct comparison of all samples focused in a single experiment. Good reproducibility was obtained with the gels that we poured ourselves, even with different lots of Immobiline buffers as well as with the commercially available gels of different lots.

We used 1,4-dioxane as the initial solvent for delipidation, which, to our knowledge, has not previously been used for apoE phenotyping. This method is not only rapid, but it is also done at room temperature. It allows the preparation of the samples and focusing on the same day. In addi-

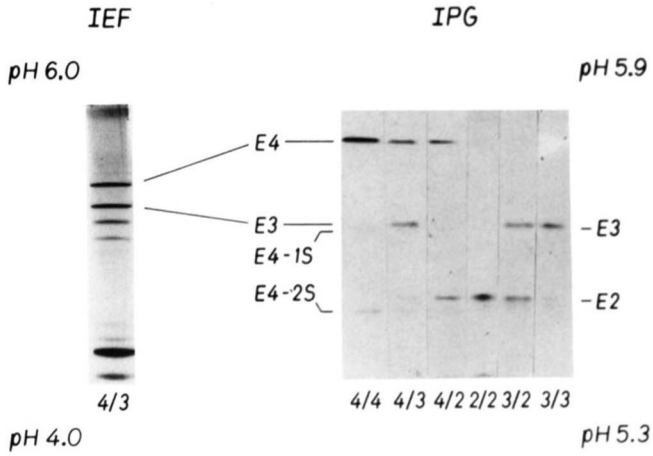

Fig. 2. Comparison of apoE isoform patterns obtained by conventional isoelectric focusing (pH 4.0–6.0 gradient) (IEF) or Immobiline isoelectric focusing (pH 5.3–5.9 gradient) (IPG) of delipidated VLDL followed by Coomassie blue R250 staining. Arrows indicate the position of E4-1S and E4-2S, which are now resolved (IPG). The immobiline gel was rehydrated with a solution of 8 M urea, 1.5% Ampholines pH 5–7 (LKB).

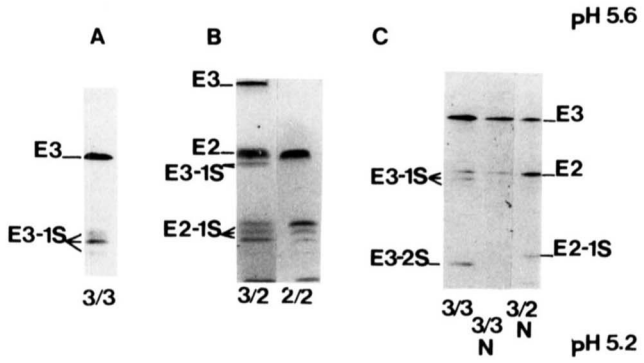

Fig. 3. Focusing of VLDL apolipoproteins in an Immobiline gel with expanded pH gradient (pH 5.2–5.6), revealing the heterogeneity of the monosialo forms. Note that the E4 isoform does not focus in this gradient but rather runs off to the cathode. The rehydration solution and the visualization procedure were the same as in Fig. 2. Panel A, apoE3/3 showing E3-1S heterogeneity; panel B, apoE3/2 and E2/2 showing E2-1S heterogeneity; panel C, neuraminidase treatment (N) of sialo heterogeneity and susceptibility to neuraminidase as in conventional IEF.

tion, dioxane does not solubilize apoB (27), thus explaining, in part, the reduced precipitation at the application point. As previously shown by Atland et al. (33), the addition of carrier ampholytes improved entry of the sample into the gel matrix and resulted in sharper band patterns. Their use in IPG analytical procedures is now recommended because of the recently discovered ability of basic Immobiline monomers to form short oligomers (34). These are thought to bridge together several proteins through hydrophobic and ionic interactions which result in precipitation at application points, as well as to promote interaction with the matrix during focusing which results in streaking patterns (33, 35). Carrier ampholytes prevent this process apparently by coating the proteins and oligomers. We found, however, as others have, that the addition of carrier ampholytes in the commercial dried gels cause liquid exudation at the surface of the gel and resulted in blurred band patterns. This effect was prevented by adding 5% Dextran T-10 to the rehydration solution (23), although the rehydration time was slightly longer.

The method was well suited for phenotyping using very small amounts of total plasma. For this purpose, the pH 5.0–6.0 commercial dried gels, which only require rehydration prior to focusing, were used. The thinness of the gel, combined with the very sensitive immunological detection method, allowed us to perform short, passive transfers of the focused proteins without significant loss of resolution. The use of neuraminidase to discriminate between E3/2 and E3/3 phenotypes proved valuable, as the E3-1S and E2 forms comigrate in this pH range. Detection of a weak signal at the monosialo position for the E3/3 phenotype was probably due to deamidation of the asialo isoform. This process is known to occur in aged or treated samples (36). In addition to the fact that such a method is ideal for routine phenotyping (owing to the minute amount of material required), it also insures correct results if, for example, one of the isoforms does not stably bind to the VLDL particles or if one of the apoE alleles is much less active (10). The amount of VLDL sample required for accurate determination of phenotypes by direct staining was also markedly decreased.

We estimated the isoelectric points of the three common apoE isoforms in the IPG gels of pH 5.0–6.0 assuming that the gradient was linear. Our estimates were approximately 0.25 pH unit below the reported values (37). We attribute this difference to a shift in the gradient towards the cathode due to the chaotropic action of urea; Immobiline monomer solutions show linear increase in their pH in the presence of urea (38).

We then accounted for this apparent shift in the design of the narrower gradients and achieved the separation of E4-1S and E3 forms and the partial resolution of the E3-1S and E2 forms. In the latter case, we uncovered the existence of multiple sialo E3 forms and found similar heterogeneity at the level of the E2-1S forms. While the uppermost sialidase-resistant band of the triplet may, as mentioned previously, have been produced by deamidation of the asialo form, the two remaining bands were sialidase-sensitive and certainly represent true sialo forms. It remains to be determined whether these are degradation intermediates generated in the circulation or reflect the attachment of the sialic acid residue through differing linkages on the single O-linked oligosaccharide of apoE. The synthesis of O-linked sugars in the Golgi apparatus is only partially understood, but it is known that several transferases having different linkage specificities exist and their relative activities vary from tissue to tissue (39, 40). We also distinguished apoE2** (Lys_{146} → Gln) from the common E2 form (Arg_{158} → Cys). Although these pairs of isoproteins bear the same total electric charge, they are nevertheless separated by IEF in IPG. A potential explanation is based on the hypothesis that even neutral amino acid substitutions affect the hydrophobicity in this seg-

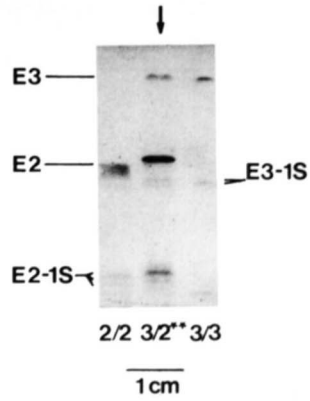

Fig. 4. Resolution of normal apoE2 and mutant apoE2** (Lys_{146}–Gln) in Immobiline gel of pH 5.2–5.6. Rehydration solution and visualization as in Fig. 2. The apoE2** mutant protein (4 μg) was resuspended in sample buffer prior to focusing.

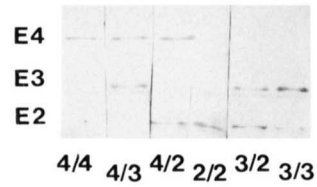

Fig. 5. Immobiline isoelectric focusing of delipidated plasma samples, which represent the six apoE phenotypes in LKB commercial dried gels (pH 5.0–6.0), after neuraminidase treatment. Rehydration conditions as in Fig. 1. ApoE isoforms bands were visualized after passive tranfer of the proteins to a nitrocellulose membrane, followed by a two-step immunoreaction: *1*) incubation with a mouse monoclonal antibody, 6C5; *2*) incubation with a rabbit anti-mouse antibody conjugated with peroxidase and incubation with a substrate solution, 4-chloro-1-naphthol. After visualization, the blot from a representative experiment with all phenotypes present was cut into strips which were rearranged for clarity in the order of presentation.

ment of the protein and modify slightly the pK of vicinal charged amino acids in native (41) or denatured proteins (42). Since the IPG system allows the use of ultra-narrow, stable gradients that have high resolution even in the presence of denaturants such as 8 M urea, it is particularly well suited to detect such closely related proteins. Whitney et al. (20) have reported the separation of mouse hemoglobin chains with neutral amino acid substitutions in IPG. Atland, Hackler, and Rossmann (23) have separated a fetal hemoglobin mutant with a Gly for Ala substitution at position 136, and have also reported the resolution of four (neutral) variant transthyretin proteins associated with the fatal familial amyloidotic polyneuropathy (21). All substitutions occurred next to or very close to charged amino acids and were detected in IPG gels. Therefore, it is not surprising that separation of the apoE isoproteins was also achieved, since these involve the removal of charged amino acids and/or the addition of a negatively charged sialic acid residues, both of which are expected to modify the pKs of nearby amino acids. It is also expected that several of the other known apoE variants, such as apoE2 Christchurch (5), apoE2* (6), and apoE3 Leiden (10) could be resolved in IPG gels. As for the incompletely resolved forms, it is possible that the gradient was not narrow enough. Further, although they have not yet been described at the protein level, it is expected that apoE proteins with truly neutral amino acid substitutions do exist and could be detected with this sensitive IPG system. These mutations may be of significance for the maintenance of cholesterol and triglyceride homeostasis. Lalazar et al. (43) have reported recently the effects on binding affinity of amino acid substitutions in the receptor binding domain of bacterially engineered apoE mutants. In one instance, the replacement of a critical proline residue with another neutral amino acid strongly reduced the binding affinity of the modified protein. Therefore, it may be useful to screen for such mutations in individuals with a family history of hyperlipidemia, using ultra-narrow gradients in selected ranges. In addition, it is likely that some of the mutations detected will not involve the receptor-binding domain. This might shed light on other functions of the apoE protein (44), which are not well understood as yet.

In summary, we have succeeded in separating the three major apoE isoforms as well as all sialo forms except for the E3-1S and E2 forms which were only partially resolved. We also resolved a known apoE2 mutant from the common E2 isoform. Therefore, we conclude that IEF in IPGs will be a useful tool in the further assessment of the genetic and post-translational variation of apoE.

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