Analysis of high density lipoprotein apolipoproteins by capillary zone and capillary SDS gel electrophoresis

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Abstract We describe new methods for analyzing the apolipoproteins (apo) of the high density lipoproteins (HDL) of several species by two modes of capillary electrophoresis: size separation using a molecular sieving buffer, and capillary zone electrophoresis (CZE) using neutral coated capillaries. By either mode HDL apos were resolved within 25 min. Results for apoA-I and apoA-II mass agreed with those by electroimmunoassay; intra-assay coefficients of variation were 1.8–4.2%. The migration times of human, rat, rabbit, and bovine apoA-I during CZE were proportional to their net charge/M_r ratios. This enabled human and rabbit apoA-I to be quantified simultaneously in transgenic rabbit HDLs. CZE also resolved human apoA-I isoforms, deamidated apoA-I, and pro-apoA-I.**—Stocks, J., M. N. Nanjee, and N. E. Miller.** Analysis of high density lipoprotein apolipoproteins by capillary zone and capillary SDS gel electrophoresis. *J. Lipid Res.* 1998. **39:** 218–227.

Supplementary key words apolipoprotein A-I • apolipoprotein A-II • sodium dodecyl sulfate • zone electrophoresis • bovine • immunoelectrophoresis • rabbit • rat • pro-apolipoprotein A-I

The high density lipoproteins (HDL) are a family of protein–lipid complexes that play a central role in reverse cholesterol transport and are a risk factor for atherosclerosis (1). Human HDLs collectively contain apolipoproteins (apo) A-I and A-II as their major protein components, together with apoC, E, and A-IV (2). In animals such as rats, in which they are the predominant lipoproteins, the HDL contain more apoE and A-IV than do human HDL. Changes in HDL composition occur during normal metabolism, as the result of genetic manipulations, and as a consequence of disease (1–3). The protein composition of HDL can be analyzed by chromatography, electrophoresis, or immunoassay. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) resolves proteins according to molecular size, while charge separation can be achieved by zone electrophoresis or isoelectric focusing. Quantification of protein bands after electrophoresis is achieved by immunoblotting or by staining with Coomassie Blue. However, due to differences in the chromogenicity of different apolipoproteins, the staining methods are only semi-quantitative (4, 5). Immunoassays offer good sensitivity and precision, but results can vary with different antisera. Furthermore, immunoassays do not distinguish between different apo isoforms.

The recent availability of high performance capillary electrophoresis (HPCE) systems has created the possibility of applying this technology to lipoprotein analysis. Protein separation by HPCE is performed at high field strengths in microbore capillaries, with direct monitoring by on-line UV detection (6). The separation is analogous to that achieved with slab gel systems, except that no support media are used, electrophoresis being carried out in free solution, and results are obtained in minutes rather than hours. Separations based on differences in molecular size are performed in an SDS-containing UV transparent polymer network. This mode, usually termed capillary SDS gel electrophoresis (though it actually uses a non-gel matrix), has been found to give results similar to those obtained with SDS slab gel electrophoresis (7, 8). Capillary zone electrophoresis (CZE) achieves separation primarily on the basis of differences in net charge/mass ratio. In this report we describe the application of both modes of HPCE to the analysis of the major apolipoproteins of human and animal HDL.

Abbreviations: HPCE, high performance capillary electrophoresis; apo, apolipoprotein; HDL, high density lipoprotein; CZE, capillary zone electrophoresis; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; TMU, tetramethylurea; i.d., internal diameter.

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METHODS

Equipment

A Beckman P/ACE 5510 system (Beckman Instruments, Fullerton, CA) fitted with a diode array detector was used. The instrument was equipped with capillary cooling and with automatic capillary rinsing and sample injection. Operation of the electrophoretic separation and the data processing of electropherograms were performed by the instrument software (System Gold).

Reagents and materials

Neutral coated capillaries for CZE (eCAP) and the coated capillaries and gel buffers for SDS electrophoresis (SDS 14-200 kit) were obtained from Beckman. Pure apoA-I and apoA-II from human plasma were purchased from Perimmune Inc. (Rockville, MD). Recombinant pro-apoA-I was provided as a gift by UCB Pharma (Brussels, Belgium). Molecular mass standards were obtained from Bio-Rad Laboratories (Hercules, CA; cat. no. 161–0317). Tricine buffer and 1,1,3,3-tetramethylurea were obtained from Sigma (Poole, Dorset, UK). Polyclonal goat antisera to human apoA-I and apoA-II were purchased from International Immunology Corp (Murrieta, CA). Precinorm L® was obtained from Boehringer-Mannheim (Lewes, Sussex, UK). All other chemicals were obtained from Fisons Scientific Equipment (Loughborough, UK). Amino acid sequences for human, bovine, rat, and rabbit mature apoA-I (9–12) were obtained from the Swiss-Prot Database, and net charge and M_r were calculated by the EGCG program, Pepstats, courtesy of HGMP Resource Centre (Cambridge, UK).

Isolation of HDL

Blood samples from non-fasting healthy humans, New Zealand White (NZW) rabbits, and NZW rabbits expressing the human apoA-I gene (13) were obtained by venipuncture, and anticoagulated with Na_2EDTA (final conc, 1 mg/ml). Male Wistar rats fed ad libitum were bled by heart puncture into Na_2EDTA (1 mg/ml). Clotted bovine blood was obtained from a local slaughterhouse on the day of collection. Plasma or serum was separated by centrifugation at 1,000 *g* for 20 min and stored at 4°C. The HDL fraction was prepared on the same day by the single vertical spin technique (14). Two ml of plasma was adjusted to a density of 1.30 g/ml by addition of solid KBr, and 1.5 ml of the solution was placed in a 5-ml Quickseal® ultracentrifuge tube (Beckman), overlayered with 0.19 mm NaCl–1.0 mm Na_2EDTA (d 1.006 g/ml), and centrifuged in a Beckman NVT90 rotor at 90,000 rpm (585,000 *g*) for 75 min at 20°C. The HDL fraction was removed using a tube-slicer or by direct aspiration, and dialyzed at 4°C against 100 mm NaCl–1.0 mm Na_2EDTA. The HDL preparations were then stored in aliquots at −80°C.

Capillary electrophoresis

Both capillary SDS gel electrophoresis and CZE were performed at 20°C with the instrument in reverse polarity (i.e., cathode at detector end of the capillary). Migration of separated proteins past the detector was monitored by absorbance at 214 nm. The chromatographic data were collected at a rate of 4 Hertz, and analyzed by the System Gold software to produce corrected migration times, peak areas, and corrected peak areas.[2] Problems with lipid deposition in the capillaries were not encountered. Each eCAP neutral capillary lasted more than 50 runs, and each SDS capillary more than 100 runs.

Capillary SDS gel electrophoresis. Preparations of HDL were either first delipidated with ethanol–ether (15) or used directly after heating with SDS sample buffer. For organic solvent delipidation 50 μl dialyzed HDL was placed in a 1.5-ml polypropylene screw-capped tube, and 1 ml ethanol–diethyl ether 2:1 (v/v; pre-cooled to −20°C) was added. The tube was vortexed and maintained at −20°C for 2 h. After addition of 0.4 ml diethyl ether, the tube was centrifuged at 10,000 *g* for 10 min at 4°C. The protein pellet was washed with diethyl ether, dried briefly, and then stored at −80°C. For electrophoresis the apoHDL (25–100 μg protein) was dissolved in 50 μl SDS sample buffer (120 mm Tris-HCl, pH 6.6, containing 1% w/v SDS) and 50 μl water. After addition of 10 μl Orange G marker (0.1% w/v) and 5 μl mercaptoethanol, the tubes were heated for 5 min at 99°C, cooled to 6°C, and centrifuged at 13,000 *g* for 5 min. Forty μl of the supernatant was then placed in a microvial prior to injection.

For quantification of HDL apolipoproteins without organic solvent extraction, HDL was denatured in SDS buffer containing human apotransferrin as internal standard. SDS sample buffer (450 μl), 50 μl apotransferrin (5 mg/ml), and 50 μl Orange G (0.1% w/v) were mixed. For separations under disulfide reducing conditions, 25 μl mercaptoethanol was also included. Twenty-five μl of this mixture was added to 25 μl dialyzed HDL (protein, 0.1–1.5 mg/ml), and the mixture was then heated, cooled, and centrifuged as described above. Areas of the apolipoprotein peaks relative to the peak area of the apotransferrin were used to calculate concentrations.

[2]Corrected migration times are the actual migration times of the components in a run, corrected for the variation in the migration time of the Orange G marker in that run. Corrected peak areas are the peak areas corrected for the velocities of the components.

Pure human apoA-I was used for calibration. Pure apolipoproteins and protein molecular mass markers were dissolved in 10 mm Tris-HCl, pH 8.0, at a concentration of 1–2 mg/ml, and samples were stored at −80°C. For electrophoresis, aliquots were transferred to 0.5-ml microtubes and 50 μl SDS sample buffer was added.

The capillaries used were Beckman coated SDS capillaries, 37 or 47 cm long (30 or 40 cm to the detector) × 100 μm internal diameter (i.d.). They were rinsed at high pressure (138 kPa) for 2 min with 1 m HCl and for 2–5 min with SDS gel buffer before each run. Cathode and anode electrolytes were SDS gel buffer. Samples were injected automatically by low pressure (3.8 kPa)

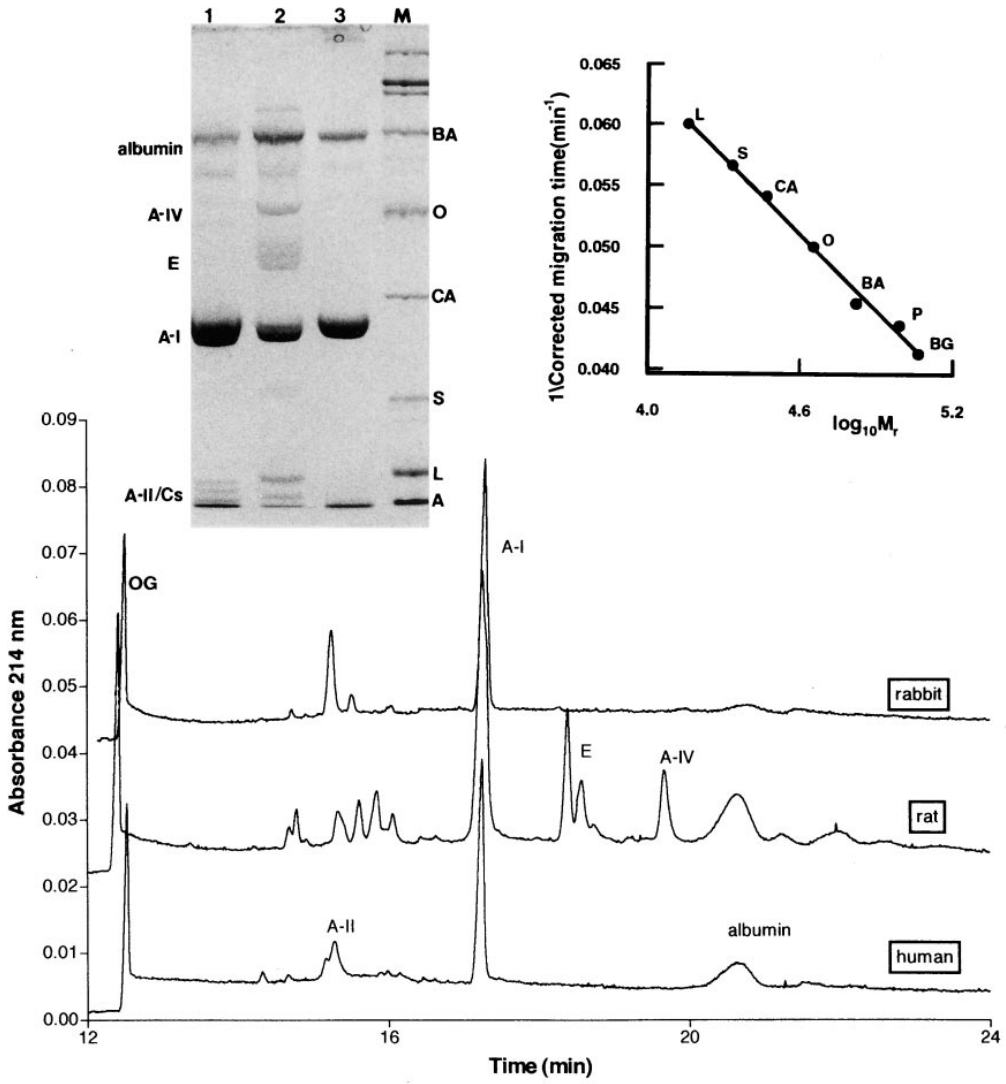

Fig. 1. Capillary SDS gel electropherograms of human, rat, and rabbit HDL apolipoproteins. The HDLs were delipidated with ethanol–ether, dissolved in SDS sample buffer containing mercaptoethanol, and heated. Samples were injected into a 47 cm × 100 μm i.d. coated capillary filled with a sieving buffer. Apolipoproteins were identified from the migration times of denatured protein molecular mass markers (upper right panel), and in the case of apoA-I and A-II by reference to the mobilities of pure standards. The lower molecular weight material in rat apoHDL was assumed to be apoA-II and apoCs. OG, Orange G; A, aprotinin (M_r 6,500); L, lysozyme (M_r 14,400); S, soybean trypsin inhibitor (M_r 21,500); CA, carbonic anhydrase (M_r 31,000); O, ovalbumin (M_r 45,000); BA, bovine serum albumin (M_r 66,200); P, phosphorylase B (M_r 97,400); BG, beta-galactosidase (M_r 116,250). Aprotinin is not shown in the plot as it fell outside the separating range of the polymer. Results obtained by SDS slab gel electrophoresis of the same HDL apolipoprotein preparations and markers, stained with Coomassie Blue, are shown for comparison (upper left panel). Lanes: 1, rabbit; 2, rat; 3, human; M, markers.

for 10–60 sec (volume, 1–5 nl), and a voltage of 11 or 14 kV (300 V/cm), ramped over 0.5 min, was applied.

Capillary zone electrophoresis. HDL preparations were delipidated either with ethanol:ether as already described or with tetramethylurea (TMU). For the latter procedure, 25 μl HDL was mixed with 25 μl TMU containing 10 mm dithiothreitol and Orange G (0.1%, w/v). The samples were incubated at room temperature for 30 min and then centrifuged. For electrophoresis apoHDL (25–100 μg) was dissolved in 50 μl 10 mm Tricine-HCl, pH 8.0, containing 6 m urea and 10 mm dithioreitol, and 5 μl Orange G marker was added. The mixture was centrifuged, and the supernatant was transferred to a microvial. For calibration of the procedure, pure human apoA-I (10–50 μg) was similarly treated.

Electrophoresis was performed in 27 or 37 cm × 50 μm i.d. eCAP neutral capillaries. These were rinsed for 1 min with 0.1 m HCl, followed by a rinse for 3 min with Tricine–Tris–urea buffer (50 mm Tricine–50 mm Tris, pH 8.0, containing 6 m urea). Cathode and anode electrolytes were 50 mm Tricine–50 mm Tris, pH 8.0. Samples were injected by pressure for 5–10 sec, and a voltage of 11 or 15 kV (400 V/cm), ramped over 0.5 min, was applied.

Immunoassays of apoA-I and A-II

ApoA-I and A-II in HDL samples were quantified without prior delipidation by rocket immunoelectrophoresis (16), using gels containing 0.1% (v/v) Tween 20 to ensure complete exposure of cryptic epitopes. Dilutions of Precinorm L® were used to prepare standards. The manufacturer's assigned values for apoA-I and apoA-II content were used for calibration.

SDS polyacrylamide slab gel electrophoresis

Conventional slab gel SDS PAGE (17) was performed in 20 × 20 cm vertical gels with 15% w/v polyacrylamide, and stained with Coomassie Blue R250 in methanol–water–acetic acid 45:45:10 (v/v/v).

RESULTS

Capillary SDS gel electrophoresis

We examined capillary SDS gel electrophoresis to establish whether size fractionation using a polymer net-

TABLE 1. Performance characteristics of the analysis of human apoA-I and apoA-II by capillary SDS gel electrophoresis and capillary zone electrophoresis

	Corrected Migration Time	Peak Area	Peak Area Relative to Internal Standard
	min	*arbitrary units*	
Capillary SDS gel electrophoresis			
Delipidated HDL			
ApoA-I	13.41 ± 0.067 (0.50%)	2.47 ± 0.108 (4.4%)	
ApoA-II	11.78 ± 0.028 (0.24%)	0.66 ± 0.033 (5.0%)	
Pure apolipoprotein			
ApoA-I	3.20 ± 0.050 (0.38%)	2.17 ± 0.102 (4.7%)	0.423 ± 0.010 (2.4%)
ApoA-II	11.67 ± 0.042 (0.36%)	2.32 ± 0.131 (5.7%)	0.396 ± 0.013 (3.3%)
Whole HDL			
ApoA-I	13.21 ± 0.058 (0.44%)	3.06 ± 0.116 (3.8%)	0.820 ± 0.021 (2.6%)
ApoA-II	11.90 ± 0.061 (0.51%)	0.89 ± 0.067 (7.5%)	0.240 ± 0.010 (4.2%)
Capillary zone electrophoresis			
Delipidated HDL			
ApoA-I	12.78 ± 0.009 (0.07%)	4.99 ± 0.089 (1.78%)	
ApoA-II	13.95 ± 0.018 (0.13%)	1.54 ± 0.030 (1.95%)	

For SDS capillary gel electrophoresis, HDL delipidated with ethanol–ether were dissolved in SDS sample buffer. Pure human apoA-I (0.5 mg/ml), pure apoA-II (0.5 mg/ml), and whole human HDL were treated with SDS sample buffer containing apotransferrin as internal standard. Separation was performed in a 37-cm capillary. For capillary zone electrophoresis, HDL apolipoproteins delipidated with TMU were separated using a 27-cm capillary in Tricine–Tris–urea buffer. The electropherograms were analyzed by the system software. Migration times were corrected for variations in the mobility of the Orange G marker. Each result is the mean ± SD (coefficient of variation) of values obtained from nine replicate injections of the same solution.

work as a sieving buffer could resolve the HDL apolipoproteins of different species with or without prior organic solvent extraction. Using apoHDL prepared by delipidation with ethanol–ether, all major apolipoproteins and albumin were resolved within 25 min (**Fig. 1**). The migration times of apoA-II, apoA-I, and albumin through a 47-cm capillary were typically 15, 17, and 21 min, respectively. The migration times of human, rat, rabbit, and bovine HDL apoA-I, pure human apoA-I, and recombinant human pro-apoA-I were identical. Electrophoresis of denatured protein size markers under the same conditions showed that their corrected migration times were linearly related to $\log_{10} M_r$ over the range 14,000–116,000 (Fig. 1, insert). The corrected migration times of the apolipoproteins were in agreement with their known molecular masses. Figure 1 also shows that the peaks seen in the capillary SDS gel electropherograms of HDL apolipoproteins corresponded to the main bands seen on slab gel SDS PAGE of the same preparations. Repeated analyses of human apoHDL and purified human apoA-I showed good precision for both the corrected migration times and the integrated areas of the protein peaks (**Table 1**).

Human HDL apoA-I and apoA-II were also quantified after heating the HDLs with SDS sample buffer without prior ethanol–ether delipidation. As longer injection times were now required, owing to the greater viscosity of the separating buffer, an internal standard of apotransferrin was included in the sample buffer to correct for variations in the volume injected. This improved the precision of the analyses and was adopted routinely (Table 1).

Electropherograms obtained using apotransferrin with or without a disulfide reducing agent (mercaptoethanol) in the SDS sample buffer are shown in **Fig. 2**. In the presence of mercaptoethanol, an additional peak migrated ahead of and overlapped that of the apoA-II monomer. Diode array scans of the faster migrating component showed that this had an absorbance spectrum (lambda maximum, 234 nm) similar to that of peroxidized phospholipid, indicating that it was probably a mixed micelle of oxidized lipid and SDS. When mercaptoethanol was omitted, the apoA-II dimer was clearly separated from the apoA-I peak. When pure human apoA-I or apoA-II was mixed with SDS reducing buffer containing apotransferrin, relative peak area was

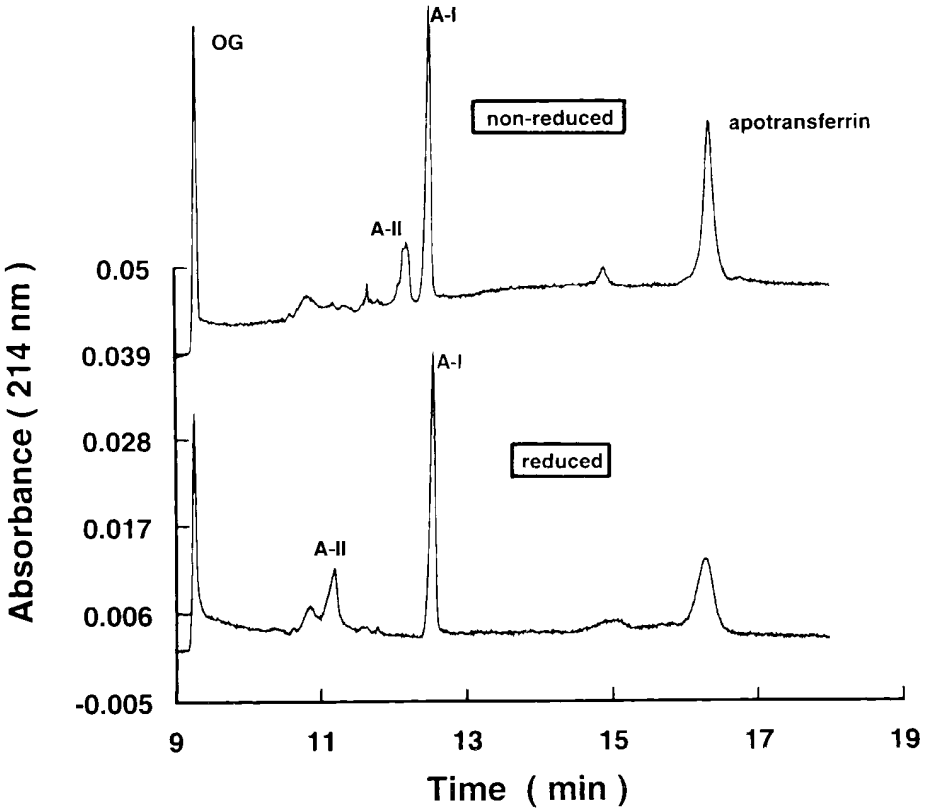

Fig. 2. Capillary SDS gel electropherograms of human HDL apolipoproteins using apotransferrin as internal standard and with (reduced) or without (non-reduced) mercaptoethanol in the SDS sample buffer. Experimental details are provided under Methods. Capillary length, 37 cm.

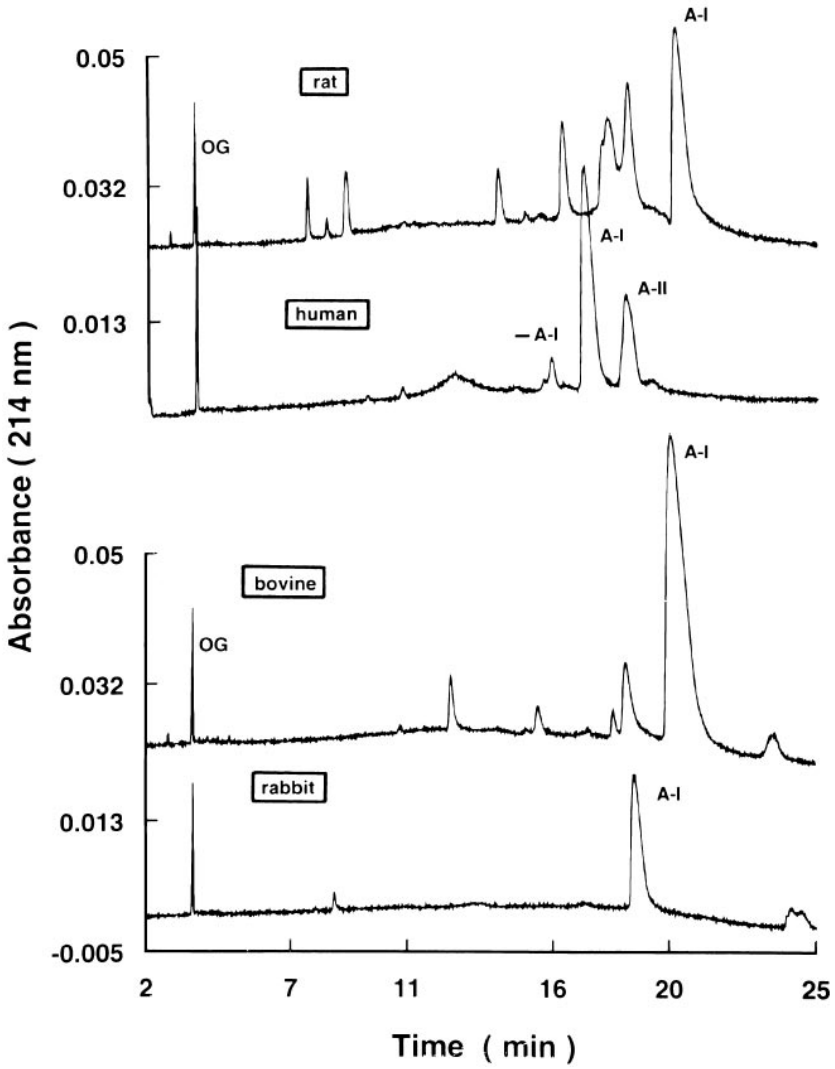

Fig. 3. Capillary zone electropherograms of human and animal HDL apolipoproteins. HDL preparations from human, rat, or rabbit plasma or bovine serum were delipidated with ethanol–ether, and electrophoresed in Tricine–Tris–urea buffer (pH 8.0) as described under Methods. Human albumin had a migration time of more than 25 min, and did not migrate past the detector under these conditions. Human apoA-I and apoA-II were identified by reference to the mobilities of pure apo, but the minor components in animal apoHDL could not be identified. Results obtained with a preparation of pure human apoA-I (not shown) suggested that the faster migrating component of apoA-I in human HDL (−A-I) was the principal deamidated form. OG, Orange G marker. Capillary length, 37 cm.

directly proportional to concentration over the range 0.1–1.0 mg/ml (apoA-I: $r^2 = 0.997$, $x = 1.263y + 0.009$; apoA-II: $r^2 = 0.985$, $x = 1.222y + 0.001$).

Capillary zone electrophoresis

Various buffers and additives were tested in an attempt to optimize resolution. Citrate buffer, pH 4.0, gave good peak symmetry, but did not adequately resolve apoA-I and apoA-II. Tricine–Tris buffer, pH 7.5–8.5, resolved apoA-I and apoA-II within 5–6 min, but gave poor peak symmetry. Electrophoresis under denaturing conditions, achieved by the inclusion of 6 m urea in the buffer, improved peak resolution, but increased the migration times to 15–20 min. Separation of human apoA-I and apoA-II in Tricine–Tris–urea was found to be optimal at pH 8.0. HDL samples delipidated with TMU produced results identical to those obtained after delipidation with ethanol–ether. Representative electropherograms of human and animal apoHDL are shown in **Fig. 3**. The electrophoretic mobilities of the major apoA-I isoforms of the different species were directly related to their net charge/M_r ra-

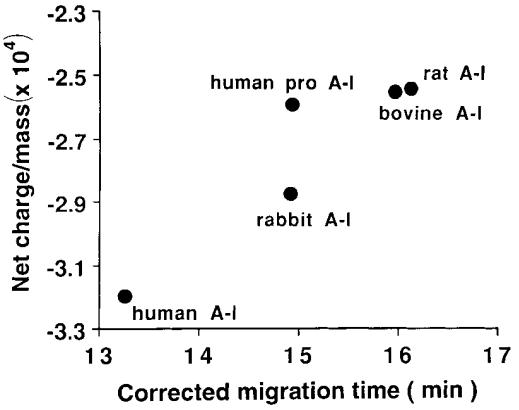

Fig. 4. Capillary zone electrophoresis: relationship of the electrophoretic mobility of HDL apoA-I from different species to net charge. Corrected migration times were determined in Tricine–Tris–urea buffer (pH 8.0). Results for recombinant human pro-apoA-I are shown. The net charge and mass of each apo were calculated from its derived amino acid composition (human apoA-I: M_r, 28,078, net charge −9; human pro-apoA-I: M_r 30,378, net charge −8; rat apoA-I: M_r 27,394, net charge −7; rabbit apoA-I: M_r 27,835, net charge −8; bovine apoA-I: M_r 27,548, net charge −7). Capillary length, 27 cm.

tios (**Fig. 4**). This enabled human and rabbit apoA-Is to be quantified simultaneously in HDLs from human apoA-I transgenic rabbits (not shown). Figure 4 also shows that recombinant human pro-apoA-I was completely resolved from the major isoform of mature human apoA-I. Good precision was achieved for both the migration times and peak areas of apoA-I and apoA-II in human HDLs (Table 1). Using pure human apoA-I and apoA-II, peak area was linearly related to concentration over the range 0.1–1.0 mg/ml (apoA-I: $r^2 = 0.979$, $x = 1.05y + 0.06$; apoA-II: $r^2 = 0.983$, $x = 1.20y + 0.05$).

Comparison with results obtained by immunoassay

Results of quantitative assays of apoA-I and apoA-II in 17 human HDL preparations by capillary SDS gel electrophoresis, CZE, and rocket immunoelectrophoresis are presented in **Fig. 5**. Good agreement was achieved.

DISCUSSION

Capillary SDS gel electrophoresis separates proteins according to differences in molecular size during migration through an entangled polymer solution, which acts as a molecular sieve (18). We have found that it can provide a convenient, rapid, and highly reproducible procedure for quantifying apoA-I and apoA-II in plasma HDL. It should also be suitable for quantifying apoE and apoA-IV in rat HDL though, as currently used, our methods do not have sufficient sensitivity to quantify these apolipoproteins in human or rabbit HDL. As quantitation of protein mass is achieved on-line by measuring absorption at 214 nm, problems of differential uptake of dye by apolipoprotein, which complicate conventional SDS gel electrophoresis (4, 19), are avoided. As sample injection is automated, it offers a potentially useful means of quantifying apolipoprotein in multiple HDL samples.

Human HDL apoA-II could not be quantified directly in monomeric form (i.e., in the presence of mercaptoethanol) by capillary SDS gel electrophoresis, owing to the apparent formation of SDS-phospholipid micelles that overlapped the apoA-II peak under these conditions, However, no such interference occurred under nonreducing conditions. Although in all but E4/E4 homozygous subjects some apoA-II in HDL is present as an apoE/apoA-II heterodimer (20, 21), this is not a problem as even in E3/E3 homozygotes less than 2% of apoA-II is present in this form (20–23). Therefore, our method is suitable for quantifying apoA-II as a homodimer in human HDL.

Some workers have used uncoated fused silica capillaries to resolve apoA-I and apoA-II in human HDL or serum by capillary electrophoresis. These techniques used SDS-containing buffers to overcome protein–capillary wall interactions, but did not use a sieving polymer. Separation depended on differences in the amount of SDS bound to individual proteins by hydrophobic interaction, resulting in differences in charge/mass ratio (24). However, these methods often gave incomplete resolution of apolipoprotein, low precision, and/or poor agreement with results by an immunoassay (25–27). Although Goux et al. (28) described the use of an SDS-polymer network to separate human apoA-I and A-II, three components migrated in the position of apoA-II, differences in the detector response to the two apolipoproteins were reported, and no comparisons were made with an independent assay.

Capillary zone electrophoresis separates proteins according to differences in net charge/mass ratio, molecular size, and shape (29). Under our conditions charge differences at pH 8.0 enabled the major isoforms of apoA-I (pl 5.65) and apoA-II (pl 4.9) to be separated. The three-fold increase in migration time which we observed, when 6 m urea was included in the buffer, probably reflected an increase in the frictional coefficient resulting from protein unfolding. In conventional slab gel zone electrophoresis (or isoelectric focusing) of apolipoprotein it is necessary to include urea or other denaturant to prevent association of the proteins (5, 30). The mobility of proteins during zone electrophoresis is

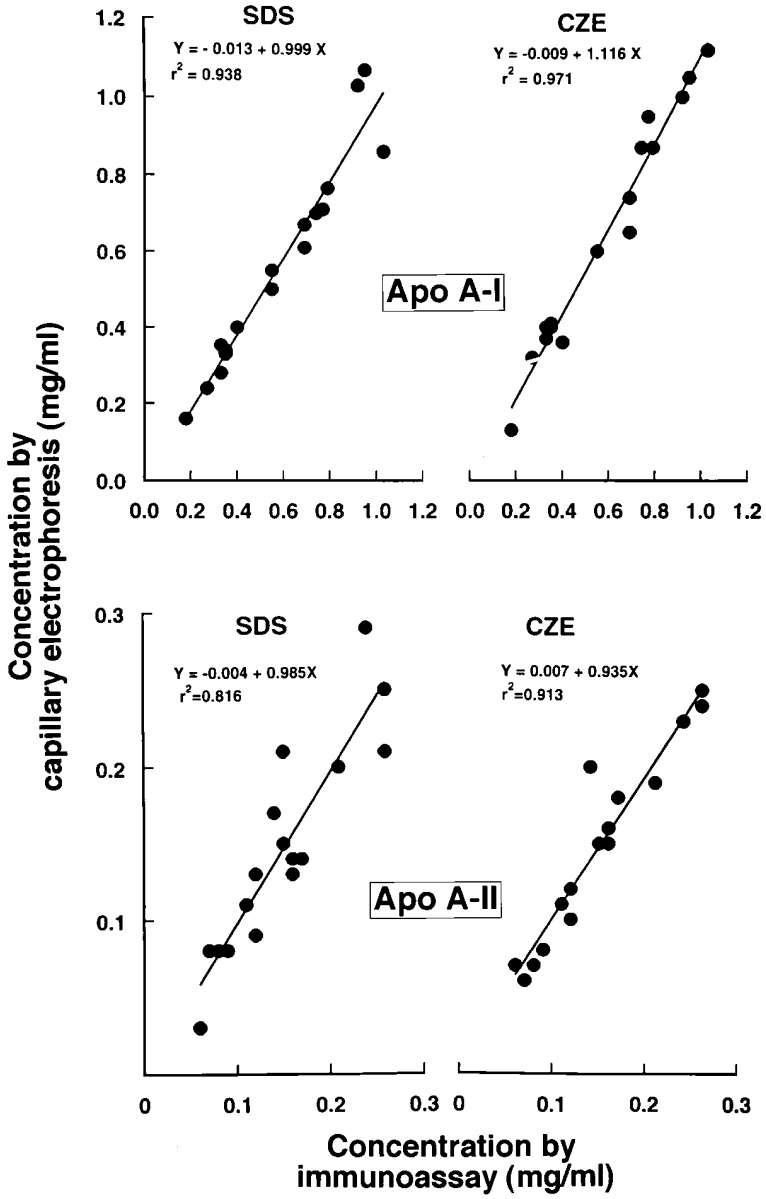

Fig. 5. Human HDL apoA-I and apoA-II concentrations measured by capillary electrophoresis and by electroimmunoassay. The apoA-I and apoA-II in HDL preparations isolated from 17 human plasmas were quantified by capillary SDS gel electrophoresis after delipidation with SDS buffer, by CZE after delipidation with TMU, and by rocket immunoelectrophoresis using a polyvalent antiserum under denaturing conditions. Results by immunoassay are the means of duplicates. The intra-assay coefficients of variation for our electroimmunoassay of apoA-I were 5.4% at 0.35 mg/ml and 2.8% at 1.38 mg/ml; for apoA-II they were 5.3% at 0.09 mg/ml and 5.3% at 0.36 mg/ml (n = 6 at each concentration). Mean concentrations (±SD) for apoA-I by immunoassay, CZE, and capillary SDS gel electrophoresis were 0.58 ± 0.26, 0.63 ± 0.29, and 0.56 ± 0.27 mg/dl, respectively. The corresponding results for apoA-II were: 0.150 ± 0.063, 0.146 ± 0.068, and 0.148 ± 0.061 mg/dl, respectively.

generally related to the ratio of net charge to $M_r^{2/3}$(31). We found that the CZE mobilities of the apoA-I of several species conformed to this rule, and that this could be exploited to quantify human and rabbit apoA-I simultaneously in transgenic rabbit HDL. Our CZE method also separated the major isoform of human mature apoA-I from human pro-apoA-I, the form in which it is secreted by hepatic and intestinal cells (32). However, it should be noted that this was demonstrated using pure recombinant pro-apoA-I. The method did not have sufficient sensitivity to quantify pro-apoA-I in normal human HDL. Using two-dimensional gel electrophoresis (33) or a specific immunoassay (34), others have reported that pro-apoA-I usually comprises only 3–7% of total apoA-I in human plasma. Other more acidic apoA-I isoforms have been reported, though these may result from deamidation or oxidation during isolation or storage (35, 36). We sometimes observed components of similar mobility during CZE of plasma-derived apoA-I, recombinant pro-apoA-I, or human HDL.

Both capillary SDS gel electrophoresis and CZE gave values for apoA-I and apoA-II concentrations in human HDL that were in good agreement with those obtained by electroimmunoassay. Thus, our methods provide rapid, reproducible, and convenient new approaches to the separation and quantification of the apolipoproteins of human and animal HDL.

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ERRATUM

In the article "Analysis of high density lipoprotein apolipoproteins by capillary zone and capillary SDS gel electrophoresis" by Drs. J. Stocks, M. N. Nanjee, and N. E. Miller, published in the January 1998 issue (*J. Lipid Res.* **39:** 218–227), Figure 4 on page 224 was inadvertently replaced by a part of Figure 2 during final page makeup. The correct Figure 4 and its legend are reproduced below. The Journal and the printer apologize for this oversight.

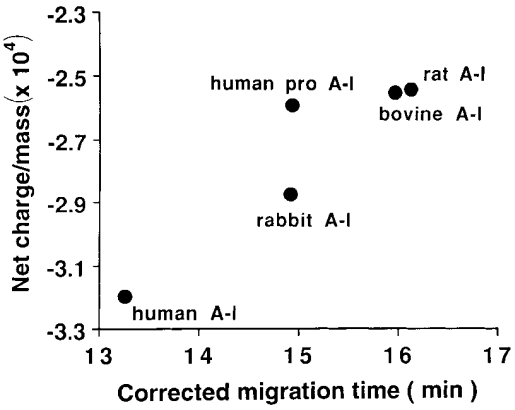

Fig. 4. Capillary zone electrophoresis: relationship of the electrophoretic mobility of HDL apoA-I from different species to net charge. Corrected migration times were determined in Tricine–Tris–urea buffer (pH 8.0). Results for recombinant human pro-apoA-I are shown. The net charge and mass of each apo were calculated from its derived amino acid composition (human apoA-I: M_r, 28,078, net charge −9; human pro-apoA-I: M_r 30,378, net charge −8; rat apoA-I: M_r 27,394, net charge −7; rabbit apoA-I: M_r 27,835, net charge −8; bovine apoA-I: M_r 27,548, net charge −7). Capillary length, 27 cm.