

Quantitative measurement of lipoprotein surface charge by agarose gel electrophoresis

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Abstract The electrophoretic mobilities of low density lipoprotein (LDL) and six pure proteins in a 0.5% agarose gel have been compared to literature electrophoretic mobility values determined by the Tiselius moving boundary method. There is a strong correlation ($r=0.99$) between the electrophoretic mobilities determined by the two techniques. The electrophoretic behavior of charged particles smaller than very low density lipoproteins (VLDL) is not markedly perturbed by a 0.5% agarose matrix, and variations in mobility primarily reflect differences in particle valence and density of surface charge. Application of electrokinetic theory to derive protein and lipoprotein net charges from the electrophoretic mobilities in agarose yields a quantitative delineation of lipoprotein electrophoretic migration patterns wherein the beta mobility region comprises a surface potential range of -4.5 to -7.0 mV; the pre-beta region a range of -7.0 to -10.5 mV; the alpha mobility region a range of -10.5 to -12.5 mV and the serum albumin region a range of -12.5 to -14.0 mV. ■ Because protein conformation and charge are critical in metabolic regulation, the agarose gel electrophoresis technique provides a valuable analytical tool that should help to elucidate further details of the structure-function relationships of serum lipoprotein particles.—**Sparks, D. L., and M. C. Phillips.** Quantitative measurement of lipoprotein surface charge by agarose gel electrophoresis. *J. Lipid Res.* 1991. **33**: 123–130.

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Over the last 20 years, electrophoresis of lipoproteins in agarose gels has been critical in lipoprotein classification and has played an important role in the characterization of a number of dyslipidemias through the development of the Frederickson, Levy, and Lees classification scheme (1). The results of lipoprotein electrophoresis in agarose have been described historically by the nomenclature beta, pre-beta, and alpha for mobilities of LDL, VLDL, and HDL, respectively (2). This nomenclature was originally formulated to describe the electrophoretic mobilities of serum proteins in moving boundary (Tiselius) electrophoresis (in free solution) and subsequently was used to describe the mobilities of different lipoprotein subfractions that comigrated with specific serum proteins (3). The complexity of the Tiselius technique eventually prompted

the development of simpler electrophoretic techniques that could reproduce the moving boundary lipoprotein electrophoretic patterns. Initially, the paper electrophoretic technique (4) became the acceptable alternative, but in 1968 Noble (2) developed an agarose electrophoretic technique that showed greatly improved resolution of the individual lipoprotein bands relative to the paper technique. Since that time, lipoprotein electrophoresis in agarose has become commonplace.

While agarose electrophoresis has been of major clinical importance, the procedure has had only minimal application as a quantitative technique. One reason for this may have been due to the ambiguity of the mobility nomenclature. Since the terms alpha and beta in agarose electrophoresis describe the relative mobilities of HDL and LDL in very general terms, this nomenclature cannot be used to report slight variations or differences in electrophoretic behavior. As such, abnormal or unusual lipoprotein electrophoretic profiles often have received qualitative names such as “sinking pre-beta VLDL” or “pre-beta HDL.” These names are of descriptive value only and may in fact be somewhat arbitrary, such as in the case of “pre-beta HDL,” where the term actually describes a group of HDL subclasses that have variable migration patterns between the pre-beta and alpha positions (5,6). Since the traditional mobility nomenclature has not allowed for the quantitative evaluation of variations in agarose electrophoretic mobility, little import has been placed upon these observations. Some studies, however, suggest that an altered agarose electrophoretic mobility of LDL from hypercholesterolemic subjects may represent a modified charge that may be central to an abnormal metabolism of these particles (7, 8). Moreover, recent

Abbreviations: LDL, low density lipoprotein; VLDL, very low density lipoprotein; HDL, high density lipoprotein; POPC, 1-palmitoyl, 2-oleoyl phosphatidylcholine; VHDL, very high density lipoprotein; rHDL, reconstituted HDL; FC, free cholesterol.

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studies indicate that the very different roles that the "pre-beta" HDL subclasses may have in cholesterol metabolism may also be associated with variations in agarose electrophoretic mobility (5, 6). If these differences in mobility indeed reflect variations in charge, their evaluation may be significant in terms of elucidating the various interactions between lipoproteins and either proteoglycans, cellular receptors, or plasma proteins involved in lipoprotein metabolism. Consequently, quantitative evaluation of agarose electrophoretic mobilities may not only help to reduce the overall ambiguity associated with the agarose mobility nomenclature, but may also help to resolve the details of some of the metabolic processes involved in lipoprotein metabolism.

Another reason for the limited quantitative application of agarose electrophoresis may be due to uncertainties as to the effect of the agarose matrix on particle electrophoretic behavior. This question was directly addressed in a 1972 study where Ghosh, Basu, and Schweppe (7) characterized the effects of both agarose gel concentration and pH and ionic strength on the electrophoretic mobility of proteins and lipoproteins. They concluded that for particles less than 80 nm in diameter, a gel matrix of low agarose concentration (0.6%) had minimal physical interference on particle movement. With this in mind, they proposed that this technique may be of value in the determination of the electrical charge characteristics of proteins by the utilization of theories developed for electrophoresis in free solution.

In this report, we show that agarose electrophoresis of lipoprotein particles closely approximates their electrophoretic behavior in the absence of an agarose matrix and that variations in mobility reflect differences in particle charge. In addition, we illustrate the application of electrokinetic theory to determine the net charge of both proteins and lipoproteins from their electrophoretic mobilities in agarose. A quantitative classification of lipoprotein electrophoretic behavior based on surface potential values in millivolts is proposed.

MATERIALS AND METHODS

Chemical supplies

Human and bovine serum albumin, ovalbumin, hemoglobin (human), fibrinogen (human), and gamma globulin (human) were purchased from Sigma Chemical Co. (St. Louis, MO). 1-Palmitoyl, 2-oleoylphosphatidylcholine (POPC) was purchased from Avanti Polar Lipids (Birmingham, AL) and guanidine HCl from Bethesda Research Laboratories (Bethesda, MD). All other reagents were analytical grade.

Isolation of lipoproteins and purification of apolipoprotein A-I

Blood from normolipidemic subjects was collected into EDTA after a 16-h fast and the plasma was removed by low speed centrifugation. VLDL, HDL, LDL, and HDL₂ and HDL₃ were isolated by sequential ultracentrifugation in the density intervals 1.250–1.210, 1.210–1.063, 1.063–1.019, and < 1.006 g/ml, respectively (9). HDL₂ and HDL₃ were similarly isolated from the total HDL pool at densities 1.063–1.125 and 1.125–1.210 g/ml. All lipoproteins were recentrifuged at their upper density limit to remove any remaining plasma proteins and then were dialyzed extensively against NaCl/Tris buffer (150 mM NaCl, 10 mM Tris/HCl, 0.3 mM EDTA, and 4.6 mM NaN₃, pH 7.4). Acetylated-LDL was prepared as described by Basu et al. (10). HDL was delipidated in ethanol–diethyl ether (11) and apolipoprotein (apo) A-I was isolated by anion exchange chromatography (12) on Q-Sepharose. Prior to use, the lyophilized apoA-I was resolubilized in 6 M guanidine HCl and dialyzed extensively against NaCl/Tris buffer. Total cholesterol was determined enzymatically using a Boehringer-Mannheim kit and the manufacturer's suggested procedures. Phospholipids were determined by the method of Sokoloff and Rothblat (13) and proteins were determined by the Lowry method as modified by Markwell et al. (14).

Preparation of discoidal apoA-I complexes

The preparation of reconstituted discoidal HDL particles (rHDL) involved a modification of the procedure originally described by Bonomo and Swaney (15). A dispersion of POPC, sodium cholate (POPC/cholate = 0.74 mol/mol) and in some cases cholesterol (FC) was prepared as previously described, allowed to clear by incubation for 1.25 h at 37°C. The cholate was removed from the dispersion by incubation with hydrated Biobeads (1 g Biobeads / 2 mg sodium cholate) for 2.5 h at 4°C. After cholate removal, lipoprotein complexes were reisolated within the density range 1.063–1.21 g/ml by ultracentrifugation and then dialyzed extensively into the appropriate buffer.

Native and recombinant lipoprotein size characterization

The sizes of native and rHDL complexes were estimated by both electron microscopy and nondenaturing gradient gel electrophoresis. Negative stain electron microscopy was performed as described by Forte and Nordenhausen (16). Micrographs were photographed at an instrument magnification of 80,000 and mean particle dimensions of 100 particles were determined from each negative. Hydrodynamic diameters were estimated for HDL-sized particles by nondenatur-

ing gradient gel electrophoresis (17) on preformed 8–25% acrylamide gels (Pharmacia Phastgel). The gels were stained for protein with Coomassie Blue R350 (Phastgel Blue R) and scanned with an E-C Apparatus EC910 densitometer. Mean diameters were calculated from a quadratic equation that was derived from polynomial regression of Stokes' diameter versus the migration distances of five standard proteins: thyroglobulin (17.0 nm), apoferritin (12.2 nm), catalase (10.4 nm), lactate dehydrogenase (8.2 nm), and bovine serum albumin (7.1 nm) (17). The number of molecules of apoA-I per particle was determined by apolipoprotein cross-linking with dimethyl suberimidate (DMS) as described by Swaney (18) and SDS PAGE was performed on 8–25% acrylamide gels to determine the extent of oligomer formation.

Agarose electrophoresis

The electrophoretic mobilities (U) of proteins and lipoproteins were determined by electrophoresis on preformed 0.5% agarose gels (Beckman, Paragon Lipo kit). Samples in Tris/NaCl, pH 8 (4 μ l buffer, 6 μ g protein) were applied to gel wells and allowed to penetrate into the gel for 5 min before the electric field was applied. A Bio-Rad model 702 power supply was used to apply a voltage of 100 ± 2 volts across a gel distance of 5.5 cm. Electrophoresis was continued for 30 min at $25 \pm 2^\circ\text{C}$ in the kit barbital buffer (pH 8.6, 0.05 ionic strength). After electrophoresis, the gels were fixed in a solution of ethanol–acetic acid–water 60:10:30 (v/v/v), oven dried (80°C for 1 h) and then stained (5 min) with a 0.15% Coomassie Blue R250 solution. Gels were destained in a solution of methanol–acetic acid–water 35:25:40 (v/v/v) for about 10 min or until the background adjacent to protein or lipoprotein bands was clear and the stain intensity of the bands was uniform (band width = 1 mm). The anodic (+) end of the gel routinely remained stained due to serum albumin present in the gel matrix (2). The migration distance was measured directly from the stained gel and was the distance (± 0.5 mm) from the point of loading to the center of each stained band. Electrophoretic migration patterns observed by staining the protein moiety of each lipoprotein were essentially identical to those observed when the lipid was stained with Sudan Black B. Both apoA-I and ovalbumin were run on each gel as internal standards to correct for slight gel to gel variations in electrophoretic mobility (coefficient of variation less than 3.5% for $n = 8$).

Analysis of electrophoretic data

Electrokinetic theory has been applied to estimate colloidal particle net charge and charge density from the electrophoretic mobility (19). The electrophoretic

mobility (U) was calculated by dividing the electrophoretic velocity (migration distance/time) by the electrophoretic potential (voltage applied/gel distance = 18.2 volts/cm). The net charge of the migrating particle was determined from the electrophoretic mobility using the relationships developed by Henry (20) from the original theory of Smoluchowski (21). Abramson, Moyer, and Gorin (19) have illustrated the derivation of these formulae and show how they may be used to evaluate the electrokinetic behavior of spherical particles of any size. The net charge or valence (V) of a spherical particle is a function of its size and electrophoretic mobility and can be derived from the relationship:

$$V = (6.25 \times 10^7) U 6\pi r n (1 + kr + kr_i) / (f(1 + kr_i)) \quad \text{Eq. 1}$$

$$= (1.049 \times 10^7) U r (1 + kr + kr_i) / (f(1 + kr_i)). \quad \text{Eq. 1a}$$

V is the number of excess positive or negative charges per particle (in electronic units), the electrophoretic mobility (U) is in $\mu\text{m} \cdot \text{s}^{-1} \cdot \text{cm} \cdot \text{V}^{-1}$, r is the particle hydrodynamic radius (cm), n is the coefficient of viscosity (0.0089 poise), and r_i is the counterion (Na for a negatively charged particle) radius (2.5×10^{-8} cm). The Debye-Huckel constant, k, which is the numerical inverse of the ionic double layer thickness (cm), is calculated from the electrolyte ionic strength (I) by the following equation (19):

$$k = I^{1/2} / (3.06 \times 10^{-8}). \quad \text{Eq. 2}$$

The constant, f, is a function of the particle size and the thickness of the ionic double layer surrounding it. Using the theory of Henry (20), Abramson et al. (19) calculated values of f as a function of spherical particle size and double layer thickness. The following polynomial (equation 3) gives the dependence of f on r at a solvent ionic strength of 0.05:

$$f = (3.66 \times 10^5 r) + (-1.74 \times 10^{11} r^2) + (3.54 \times 10^{16} r^3) + (-1.8 \times 10^{21} r^4) + 0.979 \quad \text{Eq. 3}$$

The density of surface charge (C_d) in electrostatic units (esu) / cm^2 for a spherical particle can be estimated directly from the particle valence from the relationship (19):

$$C_d = V \times 4.8 \times 10^{-10} / 4\pi r^2 \quad \text{Eq. 4}$$

$$= V \times 3.82 \times 10^{-11} / r^2. \quad \text{Eq. 4a}$$

While the charge parameter that is used to characterize macromolecular particles is usually the net charge or valence, the charge characteristics of spherical particles with hydrodynamic radii larger than molecular dimensions can also be distinguished in terms of a zeta potential which is similar to the surface potential (S). The zeta potential exists at the surface of shear of a charged particle and can be estimated from

the electrophoretic mobility using the most general form of Henry's equation (19):

$$S = U6\pi\eta/D \quad \text{Eq. 5}$$

$$= U \times 19.35 \quad \text{Eq. 5a}$$

which gives the value of S in mV for a solvent dielectric constant (D) of 78.36 when U is expressed in units of $\mu\text{m} \cdot \text{s}^{-1} \cdot \text{cm} \cdot \text{V}^{-1}$ and after converting electrostatic volts to ordinary volts. It should be noted that this relationship does not account for ionic effects and does not hold for particles of molecular size where the exact distribution of mobile ions about the central macromolecular ion is unclear. However, application of the approximate relationship given in equation 5 provides a convenient formalism for describing the surface charge characteristics of lipoprotein particles; variations in electrophoretic behavior can be expressed in terms of differences in surface potential.

Several studies (reviewed in ref. 19) have shown that particle shape may directly affect the electrophoretic behavior. The perturbing effects of certain cylindrical and discoidal shapes have been characterized and shown to be dependent on the degree of asymmetry of the particle, but the exact relationship between the surface charge and electrophoretic mobility for discoidal particles in the HDL size range (ratio of major to minor diameters = 2) is not known. However, since the average difference between the valence of a molecule such as HSA in either a spherical or cylindrical (axial ratio = 5) shape is less than one electronic unit (19), the relatively small asymmetry of nonspherical HDL particles probably has a negligible effect on the particle charge characteristics. Consequently, if a uniform charge distribution for both particle shapes is assumed, it is probably reasonable to apply the equations for spherical particles to discoidal lipoprotein particles.

RESULTS AND DISCUSSION

Calculation of electrophoretic mobility

Comparison of the agarose electrophoretic mobilities of LDL and six pure proteins to literature electrophoretic mobility values determined by the Tiselius moving boundary method (22–25) revealed a highly significant relationship ($r=0.99$) between values determined by the two methods (Table 1 and Fig. 1). In addition, a strong positive relationship ($r=0.90$) was observed between reference protein pI (22) and the electrophoretic mobility determined in agarose. However, no relationship was observed between reference protein molecular weight and agarose electrophoretic mobility. This indicates that the electrophoretic behavior of charged particles is not markedly perturbed

TABLE 1. Comparison of protein electrophoretic mobilities determined in free solution and an agarose gel matrix

Protein	Agarose Mobility ^a ($\mu\text{m} \cdot \text{s}^{-1} \cdot \text{cm} \cdot \text{V}^{-1}$)		Tiselius Mobility ^b ($\mu\text{m} \cdot \text{s}^{-1} \cdot \text{cm} \cdot \text{V}^{-1}$)	Ref.
	Observed	Corrected ^c		
BSA	0.68	0.67	0.66	23
HSA	0.65	0.65	0.61	22
Ovalbumin	0.52	0.54	0.59	24
LDL	0.21	0.29	0.31	22
Hemoglobin	0.20	0.28	0.25	25
Fibrinogen	0.09	0.19	0.21	22
Gamma globulin	0.02	0.13	0.11	22

^aElectrophoretic mobility (± 0.01 (SD)) in 0.5% agarose using a barbital buffer, 0.05 ionic strength, pH 8.6 at $25 \pm 2^\circ\text{C}$.

^bReported electrophoretic mobility determined by the Tiselius moving boundary method, barbital buffer 0.1 ionic strength, pH 8.6, (except ovalbumin, pH 7.83, and hemoglobin and LDL, phosphate buffer, pH 7.8).

^cCorrected for gel retardation effects using equation 6 (see text for details).

by a 0.5% agarose matrix and that variations in mobility primarily reflect differences in valence and charge density (cf. ref. 7).

As shown in Table 1, the two proteins that appeared to be more positively charged, gamma globulin and fibrinogen, also exhibited the greatest difference between their agarose and Tiselius electrophoretic mobilities, due to a retarded mobility in agarose. In contrast, all other proteins displayed electrophoretic mobilities in agarose which, on average, differed by less

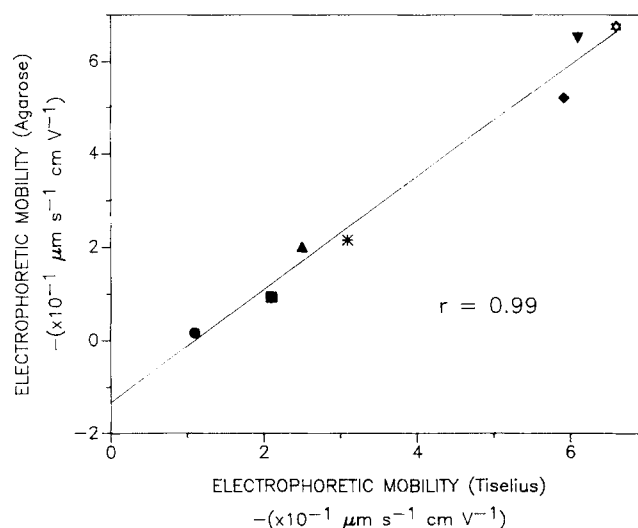


Fig. 1. The relationship between protein electrophoretic mobility determined by electrophoresis in 0.5% agarose and by the moving boundary (Tiselius) technique. Electrophoresis of LDL and six reference proteins; human serum albumin (▼), bovine serum albumin (◆), ovalbumin (♦), hemoglobin (▲), fibrinogen (■), and gamma globulin (●), showed a strong correlation between their calculated electrophoretic mobilities in agarose and those determined by the Tiselius moving boundary technique. The relationship is depicted as a regression line described by the equation: $U_{\text{Agarose}} = (1.211 \times U_{\text{Tiselius}}) + 0.136$, when U is expressed in units of $\mu\text{m} \cdot \text{s}^{-1} \cdot \text{cm} \cdot \text{V}^{-1}$.

than 10% from their mobilities in the moving boundary method. Since the agarose mobility values for the more negatively charged proteins (BSA and HSA) were almost identical to the Tiselius values, this indicates that retardation was probably not due to any electroosmotic flow (19) of the buffer solution. Furthermore, since no relationship between particle size and either electrophoretic mobility or retardation was observed in this study, it also seems unlikely that any electrophoretic retardation observed was due to a physical interference exerted by the agarose matrix itself. Comparison of the magnitudes of the differences between agarose and Tiselius electrophoretic mobilities and the protein pI values revealed a significant relationship ($r = 0.81$), wherein the higher the pI, the greater the retardation in mobility. This supports the observations of Ghosh et al. (7) showing that electrophoretic retardation in agarose was the greatest at pH values nearest the particle pI. This pI-dependent retardation effect may result from the reduced solubility of proteins at their pI, which may lead to association with residual acidic groups on the agarose matrix. Accordingly, estimation of an accurate electrophoretic mobility from agarose electrophoresis must involve correcting for this retardation effect. To account for this effect, the linear relationship between the agarose and Tiselius electrophoretic mobilities ($U_{\text{Agarose}} = (1.211 \times U_{\text{Tiselius}}) + 0.136$, Fig. 1) has been applied to resolve a corrected (Tiselius equivalent) electrophoretic mobility from the observed agarose mobility:

$$U_{\text{corrected}} = (U_{\text{Agarose}} - 0.136) / 1.211 \quad \text{Eq. 6}$$

when U is expressed in units of $\mu\text{m} \cdot \text{s}^{-1} \cdot \text{cm} \cdot \text{V}^{-1}$. As expected, correction of the reference protein electrophoretic mobilities (Table 1) results in values that have a linear relationship with the Tiselius values; the line intersects the origin and has a slope of 1. While this correction has little effect on the mobilities derived for particles in the HDL or albumin range, it substantially improves the estimates for the less negatively charged particles; for instance, in the case of LDL, the difference between the Tiselius and agarose mobilities is reduced from 32% to 7%. Even with this correction, the determination of the charge characteristics for particles with either high pI values (> 6) or mobilities less than $0.1 \mu\text{m} \cdot \text{s}^{-1} \cdot \text{cm} \cdot \text{V}^{-1}$ is difficult when measurements are made at pH 8.6 because of limitations in measuring the small migration distances.

Calculation of particle net charge

Albumins. Agarose electrophoresis of ovalbumin and two serum albumins, bovine and human, yielded electrophoretic mobilities that were almost identical to those determined by the Tiselius moving boundary technique (Table 1). Characterization of the electro-

phoretic behavior for the predominant isoform of ovalbumin gave an electrophoretic mobility in agarose of $0.54 \mu\text{m} \cdot \text{s}^{-1} \cdot \text{cm} \cdot \text{V}^{-1}$ (Table 1). When this value is used to estimate the net charge of the protein according to equations 1–3, a valence of -5.3 electronic units is computed. After correction for differences in buffer composition and temperature, this value is similar to one derived from a Tiselius electrophoretic mobility (24). This indicates that particle charge calculations derived from agarose electrophoresis data very closely approximate values based on the much more complex Tiselius technique.

As with electrophoresis in free solution (22, 23), agarose electrophoresis of the serum albumins showed both proteins to be extremely negatively charged with the mobility of BSA being slightly greater than that of HSA. This increased electrophoretic mobility for BSA reflects the fact that this protein is slightly more negatively charged with a net valence of -8.5 electronic units compared to -8.2 units for HSA. In addition, if both proteins are assumed to have similar hydrodynamic diameters (7.1 nm), similar surface charge densities of 2640 and 2560 esu/cm^2 are estimated from equation 4 for BSA and HSA, respectively.

Native lipoproteins. Fig. 2 depicts the relative migration distances of native lipoproteins, reconstituted HDL, and apoA-I electrophoresed in a 0.5% agarose gel matrix. The migration direction of all particles is toward the anodic (+) end of the gel and since particle charge is the primary determinant of electrophoretic

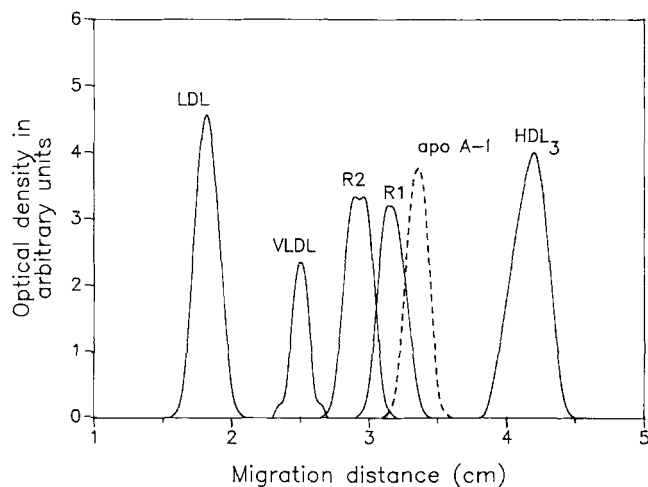


Fig. 2. Densitometer profiles showing the relative migration distances of native and reconstituted lipoproteins after electrophoresis at pH 8.6 in 0.5% agarose gels under nondenaturing conditions. The electrophoretic profiles of LDL, VLDL, and HDL₃ represent the beta, pre-beta, and alpha electrophoretic positions, respectively. The electrophoretic profiles of free apo A-I and two reconstituted discoidal complexes; R1, POPC:A-I (80:1, mol:mol) and R2, POPC:cholesterol:A-I (83:2:1) are also shown; these particles migrate to positions intermediate between the alpha and pre-beta positions.

mobility, the greater migration distance, the more negatively charged the particle. As expected, the electrophoretic patterns of LDL, VLDL, and HDL₃ are distinct and well resolved and represent the beta, pre-beta, and alpha electrophoretic positions, respectively. Electrophoretic mobility determinations for given samples of all lipoprotein classes showed a similar gel-to-gel coefficient of variation as for apoA-I (< 3.5%). Mobility values for HDL₃ isolated from six normolipidemic subjects exhibited a variability within the above limit. However, the variability observed for the electrophoretic mobilities of LDL samples from four subjects was higher (7.9%). The electrophoretic profiles of free A-I and two reconstituted discoidal complexes are also illustrated in Fig. 2; it is apparent that they migrate to points intermediate between the alpha and pre-beta positions. It is of interest to note that this is the same zone within which the small subset of more positively charged HDL called "pre-beta" HDL have been shown to migrate (26, 27). To delineate the zones associated with the various lipoprotein migration patterns in a more quantitative fashion, we propose to use the particle surface potential. Estimation of the surface potential of each lipoprotein subclass by application of equation 5 allowed us to define the regions which correspond to the customary classes of migration: the beta mobility region with a surface potential range of -4.5 to -7.0 mV; the pre-beta region with a range of -7.0 to -10.5 mV; the alpha mobility region with a range of -10.5 to -12.5 mV; and the serum albumin region with a range of -12.5 to -14.0 mV.

Electrophoretic characterization of ultracentrifugally isolated HDL subfractions illustrates some marked differences in the electrophoretic behavior of these alpha-migrating particles. Knowing the hydrodynamic radii from nondenaturing gradient gel electrophoresis (3.8, 4.5, and 5.6 nm ± 0.5 nm for VHDL, HDL₃, and HDL₂, respectively), the net charge and density of sur-

face charge of the HDL subclasses can be calculated. **Table 2** shows that the increased size of a specific HDL subclass is associated with an increased net negative charge but a decreased surface charge density. As such, the reduced electrophoretic mobility and surface potential observed for the larger HDL particles result from a significant reduction in the particle surface charge density. This is also the case for LDL and VLDL, both of which exhibit reduced electrophoretic mobilities relative to HDL and concomitantly lower surface charge densities (Table 2).

Estimation of LDL and VLDL particle charge from their estimated Stokes radii of 12.5 nm and 27.5 nm (18) and agarose electrophoretic mobilities showed these lipoprotein subclasses to have profoundly increased net negative charge (valence) relative to HDL but surface charge densities only about 50% that of HDL (Table 2). These charge characteristics may be significant in the regulation of the metabolism of low density lipoproteins. Thus, the electrophoretic behavior of LDL and VLDL isolated from hyperlipidemic subjects seems to differ substantially from that of normolipidemic subjects (7, 8). In addition, acetylation of the lysine residues of apoB-100 in LDL has been shown to increase LDL electrophoretic mobility and simultaneously impair binding to the LDL receptor (11). In this study, the change in LDL charge after acetylation has been determined and it is evident that both the valence and charge density increase by almost 50% (Table 2). Our calculations suggest that acetylation of LDL by the procedure used neutralized approximately 21 of the ionizable lysine residues in apoB because the particle valence changed from -41 to -62 (Table 2).

ApoA-I and recombinant HDL. Agarose electrophoresis of apoA-I showed the proteins to have an electrophoretic mobility of $-0.43 \pm 0.01 \mu\text{m} \cdot \text{s}^{-1} \cdot \text{cm} \cdot \text{V}^{-1}$ (Table 2). No variations in electrophoretic mobility were observed when the loading volume of apoA-I (at

TABLE 2. Electrophoretic characteristics of native and reconstituted lipoprotein particles

Complex ^a	Mobility ^b (-) $\mu\text{m} \cdot \text{s}^{-1} \cdot \text{cm} \cdot \text{V}^{-1}$	Surface Potentials ^c (-) (mV)	Charge Density ^d (-) ($\times 10^3 \text{esu/cm}^2$)	Valence ^e (-e)
ApoA-I	0.43	8.3	1.80	3.7
POPC:A-I	0.42	8.0	1.52	10.4
POPC:FC:A-I	0.40	7.8	1.48	9.9
VHDL	0.62	11.9	2.39	9.0
HDL ₃	0.59	11.5	2.22	11.5
HDL ₂	0.57	11.0	2.06	16.4
LDL	0.29	5.6	1.00	41.4
Acetyl-LDL	0.44	8.5	1.52	62.1
VLDL	0.37	7.1	1.26	249.6

^aReconstituted HDL, POPC:A-I = 80:1 (mol:mol), POPC:FC:A-I = 83:2:1 (mol:mol:mol).

^bElectrophoretic mobility in 0.5% agarose ± 0.01 (SD).

^cPotential at the surface of shear ± 0.1mV (SD) calculated using equation 5.

^dNet density of surface charge ± $0.02 \times 10^3 \text{esu/cm}^2$ (SD) calculated using equation 4.

^eThe number of excess negative charges in electronic units per particle ± 0.1 (SD) calculated using equation 1.

1.5 mg protein/ml) was varied from 1 to 5 ml. In addition, there was also no detectable change in electrophoretic mobility when the loading volume of apoA-I was kept constant (4 μ l) but the protein concentration was varied from 0.25 to 2.5 mg/ml. Since studies have shown that at concentrations greater than 0.5 mg protein/ml, apoA-I has a propensity to self-associate (for a review, see ref. 28), these results suggest that the self-association of the protein has no effect on its electrophoretic mobility. In 1980, Edelstein and Scanu (29) showed that in solution, monomeric apoA-I molecules adopt an expanded ellipsoidal shape with an estimated hydrated Stokes radius of 2.8 nm. Calculation of apoA-I charge from this hydrated radius and our estimation of the protein electrophoretic mobility gave a molecular valence of 3.7 negative charges (electronic units) and a charge density of -1.80×10^3 esu/cm² in the buffer employed (Table 2).

As shown in Fig. 2, complexing apoA-I with phospholipid resulted in the formation of a discoidal particle that contained two molecules of apoA-I per particle and approximately 80 mol of POPC per mol apoA-I. This particle was less negatively charged than apoA-I but also migrated within the proposed pre-beta zone, in a fashion similar to "pre-beta" HDL particles identified by others (26, 27). Fig. 2 shows the mobility of an identical particle reconstituted to also contain four molecules of cholesterol. Electron microscopy showed both particles to be discoidal in appearance and of similar size. Hydrodynamic particle diameters, determined by nondenaturing gradient gel electrophoresis, were similar to electron micrograph size determinations and were the same for both rHDL, $10.2 \text{ nm} \pm 0.5 \text{ nm}$. Table 2 illustrates the electrophoretic characteristics of these discoidal reconstituted lipoproteins. When apoA-I is complexed with POPC into a 80:1 (mol/mol) disc, a slight reduction in the magnitude of electrophoretic mobility and surface potential, relative to the free protein, is observed. This reduced mobility appeared to be primarily due to a decrease in the density of negative surface charge from 1.80×10^3 to 1.52×10^3 esu/cm². Since this complex contained two molecules of apoA-I per particle, the valence of apoA-I on an 80:1 discoidal complex is -5.2 electronic units per molecule of apoA-I. This valence for apoA-I associated with lipid on a disc indicates that it is more negatively charged than when free in solution where the valence is -3.7 . Electrophoretic characterization of a second complex of a similar POPC:A-I composition but with an additional four molecules of cholesterol (FC) showed that even though both particles were similar in size, incorporation of cholesterol modified the complex electrophoretic behavior (Fig. 2 and Table 2). Determination of apoA-I valence on the 83:2:1 (molar ratio of POPC-FC-A-I) discoidal com-

plex suggests that the addition of cholesterol resulted in a slight reduction of both the valence and surface charge density of the complex. The observed changes in particle charge may be a reflection of an effect of cholesterol on apoA-I conformation (30).

Conclusions

Electrokinetic analysis of the agarose electrophoretic mobilities of proteins and lipoproteins closely approximates that of the technically more complex moving boundary technique, and as such allows for an accurate estimation of particle charge. A quantitative definition of serum lipoprotein electrophoretic migration patterns in an agarose matrix is possible. Furthermore, electrophoretic mobility data obtained by this relatively simple technique can be used to resolve important molecular information about protein structure and charge. Because protein conformation and charge are critical in metabolic regulation, the agarose gel electrophoresis technique should help to elucidate further details of the structure-function relationships of serum lipoprotein particles. ■

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