Influence of the electrostatic charge of lipoprotein particles on the activity of the human plasma phospholipid transfer protein

Catherine Desrumaux, Anne Athias, David Masson, Philippe Gambert, Christian Lallemant, and Laurent Lagrost

Laboratoire de Biochimie des Lipoprotéines, INSERM CJF 93-10, Faculté de Médecine, 21033 Dijon Cedex, France

Abstract The aim of the present study was to determine the effect of the electrostatic charge of lipoproteins on the phospholipid transfer activity of the plasma phospholipid transfer protein (PLTP). Progressive decreases in the PLTP-mediated phospholipid transfer rates were observed when the surface potential of isolated high density lipoproteins (HDL) was either reduced from $-11.7$ mV down to $-15.7$ mV by succinylation of apolipoprotein (apo) A-I by apoA-I. When succinylated low density lipoprotein (LDL) series with surface potentials ranging between $-4.3$ mV and $-14.3$ mV were used, successive increase and decrease in phospholipid transfer rates were observed along the electronegativity scale. When various plasma HDL subfractions with surface potentials ranging from $-10.5$ mV to $-12.5$ mV were separated by anion exchange chromatography, PLTP-mediated phospholipid transfer activity increased progressively with HDL electronegativity until maximal lipid transfer rates were reached for a mean HDL surface potential of $-11.6$ mV. As the electronegativity of plasma HDL subfractions kept increasing beyond the optimal value, a progressive decrease in PLTP activity was observed. Striking parallelism between cholesteryl ester transfer protein (CETP) and PLTP transfer activity curves obtained with each HDL series were noted, and the optimal HDL surface potential values were remarkably similar, approximating $-11.6$ mV in all the experiments. With isolated plasma LDL subfractions with surface potentials ranging from $-3.5$ mV to $-5.0$ mV, a linear rise in PLTP activity was observed. In conclusion, data of the present study indicate that, like CETP, the activity of PLTP is influenced by electrostatic interactions with lipoproteins—Desrumaux, C., A. Athias, D. Masson, P. Gambert, C. Lallemant, and L. Lagrost. Influence of the electrostatic charge of lipoprotein particles on the activity of the human plasma phospholipid transfer protein. J. Lipid Res. 1998. 39: 131–142.

Supplementary key words CETP • PLTP • surface potential • electronegativity • HDL • LDL

In human plasma, cholesteryl esters, triglycerides, and phospholipids can exchange between various lipid protein classes through the action of two distinct lipid transfer proteins, the cholesteryl ester transfer protein (CETP), and the phospholipid transfer protein (PLTP) (1). Genes encoding CETP and PLTP present similar organizations, and recent comparisons of DNA sequences revealed that the two plasma lipid transfer proteins belong to the same gene family, i.e. the lipid transfer lipopolysaccharide binding protein (LT/LBP) family, indicating that CETP and PLTP might derive from a common ancestral gene (2-4).

Concordant observations of the last decade demonstrated that the CETP-mediated lipid transfer reaction is a complex process that is initiated by the electrostatic interaction of positively charged groups of CETP with negative charges of the lipoprotein surface (5-11; for a review, see 12). It has been shown that the interaction of CETP with modified lipoproteins is facilitated when lipoprotein negative charge is increased, and it is reduced when lipoprotein electronegativity is decreased (5, 7, 8, 11). Recent studies with plasma HDL subfractions of distinct electrostatic charge confirmed that the electronegativity of HDL particles constitutes an important determinant of human CETP activity (13), and optimal CETP-lipoprotein interactions are required for maximal cholesteryl ester transfer rates, with either insufficient or excessive amounts of electronegative charges.

Abbreviations: apo, apolipoprotein; CETP, cholesteryl ester transfer protein; esu, electrostatic unit; FPLC, fast protein liquid chromatography; HDL, high density lipoprotein; HDL$_2$, HDL subfraction 2; HDL$_3$, HDL subfraction 3; [${}^{14}$C]DPPC-HDL$_2$, HDL$_2$ containing radio-labeled dipalmitoyl phosphatidylcholine; LDL, low density lipoprotein; [${}^3$H]CE-LDL, LDL containing radiolabeled cholesteryl esters; LT/LBP, lipid transfer/lipopolysaccharide binding proteins; NEFA, non-esterified fatty acids; PLTP, phospholipid transfer protein; TBS, Tris-buffered saline.

1To whom correspondence should be addressed.
charges at the lipoprotein surface resulting in a decrease in the efficiency of the CETP-mediated lipid transfer process (11, 13). The physiopathological relevance of the role of negative charges in determining plasma CETP activity was further evidenced in hyperbrominemic plasmas in which concomitant rises in lipoprotein electronegative charges and cholesteryl ester transfer rates were observed (14).

In contrast to CETP activity, PLTP activity remains to be fully characterized, and determinants of the interaction of PLTP with lipoprotein substrates are still unknown. Based on previous studies, which reported a 20% homology between CETP and PLTP aminoacid sequences (3), there might be some similarities in the interaction of CETP and PLTP with lipoprotein particles. In order to investigate the latter hypothesis, the effect of variations in the lipoprotein electronegative charge on PLTP activity was examined in the present study. To this end, the amounts of electronegative charges in HDL and LDL surfaces were modified by succinylation of lysyl amino groups of apolipoproteins, and consequences in terms of their ability to act as substrates for PLTP were determined. As recent studies from our group demonstrated that the replacement of apoA-I by apoA-II in HDL is associated with parallel reduction in HDL electronegativity and CETP activity (13), we also studied the effect of substitution of apoA-II for apoA-I in HDL on PLTP activity. Finally, human plasma HDL and LDL were fractionated according to their electrostatic charge by using anion exchange chromatography, and the ability of various plasma lipoprotein subpopulations to serve as substrates in the PLTP-mediated transfer reaction was evaluated.

MATERIALS AND METHODS

Isolation of HDL and LDL particles

Fresh citrated plasma from normolipidemic subjects was provided by the Centre de Transfusion Sanguine (Hôpital du Bocage, Dijon, France). Low density lipoproteins (LDL) were isolated as the 1.019 g/ml plasma fraction by sequential ultracentrifugation at 100,000 g (149,000 rpm) in a 70-Ti rotor in an L7 ultracentrifuge (Beckman, Palo Alto, CA), with one 24-h spin at the density of 1.073 g/ml, at a speed of 90,000 rpm (561,000 g) in an NVT-90 rotor in an XL-90 ultracentrifuge. Densities were adjusted by the addition of solid potassium bromide (KBr). The isolated lipoproteins were dialyzed overnight against a Tris/HCl buffer (10 mmol/l Tris, 3 mmol/l Na3, pH 7.4).

Succinylation of LDL and HDL

LDL and HDL were succinylated according to a procedure previously described (15). Briefly, plasma HDL or plasma LDL (protein concentration, 1.25 g/l) were mixed with succinic anhydride (concentration range, 0–5 mmol/l) in a final volume of 325 µl in a 10 mmol/l Tris, 150 mmol/l NaCl, 1 mmol/l Na2-EDTA, 3 mmol/l Na3, pH 7.4 buffer (TBS buffer). After the succinylation reaction, pH was adjusted to 7.5 by the addition of NaOH. Resulting succinylated lipoproteins were dialyzed against TBS buffer, and the effect of succinylation in terms of lipoprotein electronegative charge was assessed by agarose gel electrophoresis.

Preparation of apoA-II-enriched HDL

ApoA-I in total plasma HDL was progressively replaced by ApoA-II upon the incubation of freshly isolated HDL in the presence of increasing amounts of delipidated HDL apolipoproteins, according to the general procedure previously described (16). The HDL to apoHDL ratio ranged from 1:0 to 1:5, allowing us to obtain HDL particles with apoA-II:apoA-I percentage mass ranging from 28.9 to 58.9%.

Fractionation of HDL and LDL by anion exchange chromatography

Freshly ultracentrifugally isolated plasma HDL and LDL were fractionated on a MonoQ HR 5/5 anion exchange column (Pharmacia Biotech Inc.) connected to a Fast Protein Liquid Chromatography (FPLC) system (Pharmacia) according to the procedures described by Masson, Athias, and Lagrost (13) and Chapp et al. (17), respectively. Approximately 20 mg of HDL protein or 2 mg of LDL protein was applied on the column, and HDL and LDL subfractions were eluted according to NaCl gradients increasing from 0 to 0.4 mol/l (13) or from 0 to 0.3 mol/l (17), respectively. In all cases, protein-containing fractions (1 ml each) were monitored at 280 nm by using an UV-1 detector (Pharmacia Biotech Inc.).

Determination of lipoprotein electronegativity by agarose gel electrophoresis

The electrophoretic mobility (U) of HDL and LDL particles was evaluated by electrophoresis on 5% agarose gel electrophoresis.
rose gels (Paragon Lipo kit, Beckman) according to the method described previously (13, 18). Mean migration distances were obtained by analysis of the gel on a Bio-Rad GS-670 imaging densitometer, with an accuracy of 0.1 nm.

Surface potentials and densities of surface charge of HDL and LDL were calculated as previously described (13, 18). Given the accuracy of the determination of electrophoretic migration distances, the mean electrophoretic potential of HDL subfractions could be calculated with an uncertainty lower than 0.1 mV.

### Native polyacrylamide gradient gel electrophoresis

Apparent hydrodynamic diameters of HDL and LDL were determined by electrophoresis in 1.5-25% nondenaturing polyacrylamide gradient gels according to the general procedure described by Blanche et al. (19). The electrophoretic migration was conducted for 1 h at 70 V, and then for 20 h at 150 V in a 90 mmol/l Tris, 80 mmol/l boric acid, pH 8.3, buffer containing 2 mmol/l Na₂EDTA and 3 mmol/l NaN₃. The gels were stained with Coomassie Brilliant Blue G. The distribution profiles of the particles were obtained by using a Bio-Rad GS-670 imaging densitometer. The mean apparent diameters of HDL were determined by comparison with globular protein standards (high molecular weight (HMW) protein calibration kit, Pharmacia Biotech Inc.) submitted to electrophoresis together with the samples. The mean apparent diameter of LDL subfractions was determined by comparison with HMW globular proteins and with carboxylated latex beads (diameter, 38 nm; Duke Scientific). The mean diameter of the subfractions was calculated from the total area under the densitometric curve, 50% of the total area being of smaller size and 50% of the total area being of larger size as compared with the mean size value. The accuracy of the determination of the mean lipoprotein diameter was 0.1 nm.

### Preparation of CETP and PLTP active fractions

CETP and PLTP were purified from fresh citrated human plasma. All purification steps were performed on a FPLC system (Pharmacia), according to the sequential procedure previously described (20). Briefly, the d>1.21 g/ml plasma proteins were isolated by a 24-h, 55,000 rpm ultracentrifugation step performed in a 70-Ti rotor in an L7 ultracentrifuge. The resulting infranatant was fractionated successively by hydrophobic interaction chromatography on a phenyl-Sepharose CL-4B column (Pharmacia), affinity chromatographies on heparin-Ultragel A4R and Blue-Trisacryl columns (Sepracor, Villeneuve-la-Garenne, France), and anion exchange chromatography on a MonoQ 5/5 column (Pharmacia). This procedure allowed us to obtain two distinct lipid transfer protein preparations, both of which were deprived of lecithin:cholesterol acyltransferase activity. Although a complete purification of CETP and PLTP was not achieved (two to four bands were visible after denaturing polyacrylamide gradient gel electrophoresis of either preparation), the resulting CETP-containing fraction did not contain PLTP, and the PLTP-containing fraction did not contain CETP (20).

### Measurement of phospholipid transfer activity

Phospholipid transfer activity was determined in TBS, pH 7.4, buffer by measuring the transfer of radio-labeled phospholipids either from radiolabeled dipalmitoyl phosphatidylcholine-containing liposomes ([¹⁴C]DPPC-liposomes) to unlabeled HDL, or from radiolabeled dipalmitoyl phosphatidylcholine-containing HDL₃ ([¹⁴C]DPPC-HDL₃) to unlabeled LDL, as indicated. When indicated, PLTP activity was also determined in barbital, pH 8.6, buffer. [¹⁴C]DPPC-liposomes were prepared according to the general procedure described by Damen, Regts, and Scherphof (21). [¹⁴C]DPPC-HDL₃ were prepared by mixing ultracentrifugally isolated HDL₃ with an ethanolic solution of [¹⁴C]dipalmitoyl phosphatidylcholine. After a 6-h incubation at 37°C, [¹⁴C]DPPC-HDL₃ were recovered by ultracentrifugation as described above, and dialyzed against TBS buffer. The transfer of [¹⁴C]DPPC from liposomes to HDL was determined by incubating for 30 min at 37°C [¹⁴C]DPPC-liposome donors (6.5 µg of phosphatidylcholine) with HDL (13 µg of phosphatidylcholine) in the presence of partially purified PLTP (25 µg) in a final volume of 40 µl. The transfer of [¹⁴C]DPPC-HDL₃ to LDL was determined by incubating for 3 h at 37°C [¹⁴C]DPPC-HDL₃ (9 µg of phosphatidylcholine) with LDL (45 µg of phosphatidylcholine) in the presence of purified PLTP (25 µg) in a final volume of 40 µl. Spon- taneous phospholipid transfers were determined simultaneously by incubating blank control samples with no purified PLTP added. At the end of the incubation, samples containing either liposomes and HDL, or HDL₃ and LDL were placed on ice, and a 35-µl volume of each sample was added to 1.95 ml of a 1.065 g/ml KBr solution in 2-ml Quickseal centrifugation tubes (Beckman). The tubes were sealed, and a 16-h, 4°C ultracentrifugation was conducted at 35,000 rpm (132,000 g) in a 50.4 Ti rotor, in an L7 ultracentrifuge (Beckman). The d<1.063 g/ml and the d>1.063 g/ml fractions were then recovered in 1-ml volumes and transferred into counting vials containing 2 ml of scintillation fluid (OptiScint Hisafe 3, Pharmacia). The radioactivity was assayed for 2 min in a Wallac 1410 liquid scintillation counter (Pharmacia). In control samples, less than 5% of the radioactivity was recovered in the acceptor lipoprotein fraction. Phospholipid transfer ac-
tivity was expressed as the percentage of radioactivity transferred from the radiolabeled donor particles towards the acceptor fractions, after deduction of blank control values which did not vary in any systematic way under the various experimental conditions used in the present study. All phospholipid transfer assays were performed in triplicate.

Measurement of cholesteryl ester transfer activity

Cholesteryl ester transfer activity was evaluated by measuring the transfer of radiolabeled cholesteryl esters from radiolabeled cholesteryl ester-containing LDL ([3H]CE-LDL) to unlabeled acceptor HDL. [3H]CE-LDL were biosynthetically prepared as previously described (16). The transfer of [3H]CE from radiolabeled donors (2.5 nmol of cholesterol) towards unlabeled lipoprotein acceptors (10 nmol of cholesterol) was determined after a 3-h incubation at 37°C in the presence of purified CETP (15 μl) in a final volume of 50 μl. Blank control samples, containing both donor and acceptor lipoproteins, were incubated for 3 h at 37°C in the absence of purified CETP. At the end of the incubation, the tubes were immediately placed on ice, and a volume of 45 μl of each incubation mixture was added to 1.95 ml of a d = 1.07 g/ml KBr solution in 2-ml Quick-seal centrifugation tubes (Beckman). The tubes were then sealed and subjected to ultracentrifugation for 16 h at 35,000 rpm in a 50.4 Ti rotor in an L7 ultracentrifuge. The d<1.068 g/ml and d>1.068 g/ml fractions were recovered in a 1-ml volume and transferred into counting vials containing 2 ml of scintillation fluid. The radioactivity was assayed for 2 min in a Wallac 1410 liquid scintillation counter. Cholesteryl ester transfer activity was expressed as the percentage of radiolabeled cholesteryl esters transferred from the lipoprotein tracer to the d>1.068 g/ml acceptor fraction. In control mixtures, containing radiolabeled LDL, less than 10% of the radioactivity was recovered in the d>1.068 g/ml fraction. In all cases, blank control values were deducted from the calculated transfer values. All cholesteryl ester transfer assays were performed in triplicate.

Protein and lipid analyses

All chemical assays were performed on a Corbas-Fara Centrifugal Analyzer (Roche). Total cholesterol and unesterified cholesterol concentrations were measured by enzymatic methods using Boehringer Mannheim reagents. Triglyceride and phospholipid assays were performed by enzymatic methods using Roche and Biomérieux reagents, respectively. Concentrations of apoA-I and apoA-II were determined by immuno-turbidimetry with anti-apoA-I and anti-apoA-II antibodies purchased from Boehringer Mannheim. Calibration serum containing apoA-I and apoA-II was purchase from Boehringer. Protein concentrations were measured with the bicinchoninic acid reagent (Pierce), according to Smith et al. (22).

RESULTS

Effect of succinylation of HDL and LDL particles on PLTP activity

In a first attempt to determine whether the electrostatic charge of lipoprotein particles can influence PLTP activity, the electronegativity of ultracentrifugally isolated HDL and LDL was artificially enhanced by incubation in presence of succinic anhydride (see Materials and Methods). Ultracentrifugally isolated plasma HDL (fraction I; protein concentration, 1.25 mg/l) and LDL were succinylated by incubation in the presence of three distinct concentrations of succinic anhydride (1.25 mmol/l, 2.50 mmol/l, and 5.00 mmol/l for fractions II, III, and IV, respectively). As shown in Table 1, succinylation of total plasma HDL led to marked and gradual alterations in their electrophoretic mobility, without affecting their mean apparent diameter. The charge characteristics of HDL were calculated from their migration distance in agarose gel and from their mean diameter by using the equations established by Sparks and Phillips (18). The surface potentials decreased from −11.7 mV in native HDL down to −15.7 mV in the most succinylated particles, and corresponding densities of electronegative charges ranged between 2.198 and 2.941 −esu/cm² (Table 1). Succinylation induced progressive decreases in the ability of PLTP to transfer phospholipids from [14C]DPPC-liposomes to HDL, and the rate of transfer of radiolabeled phosphatidylcholine decreased from 16.2% in native HDL down to 10.5% in the most electronegative particles (Fig. 1).

Table 1. Size and charge characteristics of plasma HDL after succinylation of their protein moiety

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Particle Diameter (nm)</th>
<th>Agarose Migration</th>
<th>Surface Potential (mV)</th>
<th>Density of Surface Charge (esu/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>9.4</td>
<td>24.8</td>
<td>11.7</td>
<td>2.198</td>
</tr>
<tr>
<td>II</td>
<td>9.4</td>
<td>27.9</td>
<td>12.9</td>
<td>2.422</td>
</tr>
<tr>
<td>III</td>
<td>9.4</td>
<td>32.9</td>
<td>14.8</td>
<td>2.783</td>
</tr>
<tr>
<td>IV</td>
<td>9.4</td>
<td>35.1</td>
<td>15.7</td>
<td>2.941</td>
</tr>
</tbody>
</table>

The negative charge of total HDL was artificially modified by succinylation of their apoprotein moiety, and the size and electrophoretic characteristics of modified HDL were determined as described under Materials and Methods. Data correspond to single determinations and they are representative of two distinct experiments.
When ultracentrifugally isolated LDL (fraction I; protein concentration, 1.25 g/l) were incubated in the presence of four distinct concentrations of succinic anhydride (1.25 mmol/l, 2.50 mmol/l, 3.75 mmol/l, and 5.00 mmol/l for fractions II, III, IV, and V, respectively), we again observed substantial and gradual increases in the electrophoretic mobility of the particles (Table 2). The mean surface potential decreased from 24.3 mV in native LDL (fraction I) down to 214.3 mV in the most succinylated LDL (fraction V), and corresponding densities of negative charges ranged between 629 and 2,107 esu/cm², respectively. A bimodal curve was obtained when measuring the rate of transfer of radiolabeled phosphatidylcholine from [14C]DPPC-HDL3 towards the various LDL preparations. As shown in Fig. 2, phospholipid transfer activity increased from 20.1% up to 28.7% as the surface potential decreased from −4.3 mV to −5.8 mV. In contrast, the rate of phospholipid transfer decreased down to 23.7% as the surface potential reached the value of −14.3 mV (Fig. 2).

Effect of apolipoprotein-induced alterations in HDL electrostatic charge on PLTP activity

The substitution of apoA-II for apoA-I in isolated plasma HDL provided us with an additional mean of inducing significant and controlled changes in the charge characteristics of HDL particles, without alterations in their lipid content (16, 23). As shown in Table 3, the surface potential of HDL increased from −11.6 up to −10.9 mV, and the density of electronegative charges decreased from 2,245 down to 2,096 esu/cm², as the A-II:A-I mass ratio increased from 28.9 up to 58.9%, respectively. As shown in Fig. 3, the PLTP-mediated transfer of radiolabeled phosphatidylcholine from [14C]DPPC-liposomes towards HDL progressively decreased as the surface potential increased from −11.6 mV in...

![Fig. 1. Effect of the surface potential of succinylated HDL on the rate of radiolabeled phospholipids transferred from liposomes to HDL in the presence of purified PLTP. The rates of phospholipids transferred from liposomes to HDL were determined by incubating [14C]DPPC-liposomes (6.5 μg of phosphatidylcholine) and HDL (13 μg of phosphatidylcholine) for 30 min at 37°C in the presence of purified PLTP (25 μg) in a final volume of 40 μl. At the end of the incubation, liposomes and HDL were separated by ultracentrifugation and PLTP activity was calculated as described under Materials and Methods. Each point represents the mean ± SD of triplicate determinations. Data are representative of two distinct experiments.](image1)

![Fig. 2. Effect of the surface potential of succinylated LDL on the rate of radiolabeled phospholipids transferred from HDL3 to LDL in the presence of purified PLTP. The rates of phospholipids transferred from HDL3 to LDL were determined by incubating [14C]DPPC-HDL3 (9 μg of phosphatidylcholine) and LDL (45 μg of phosphatidylcholine) in the presence of purified PLTP (25 μg) in a final volume of 40 μl. At the end of the incubation, LDL and HDL3 were separated by ultracentrifugation and PLTP activity was calculated as described under Materials and Methods. Each point represents the mean ± SD of triplicate determinations. Data are representative of two distinct experiments, and they correspond to data given in Table 2.](image2)

**Table 2.** Size and charge characteristics of plasma LDL after succinylation of their protein moiety

<table>
<thead>
<tr>
<th>Fraction Number</th>
<th>Particle Diameter</th>
<th>Agarose Migration</th>
<th>Surface Potential</th>
<th>Density of Surface Charge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nm</td>
<td>mm</td>
<td>mV</td>
<td>esu/cm²</td>
</tr>
<tr>
<td>I</td>
<td>25.8</td>
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<td>II</td>
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<td>809</td>
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<td>25.8</td>
<td>9.5</td>
<td>5.8</td>
<td>857</td>
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<tr>
<td>IV</td>
<td>25.8</td>
<td>23.5</td>
<td>11.2</td>
<td>1,651</td>
</tr>
<tr>
<td>V</td>
<td>25.8</td>
<td>31.6</td>
<td>14.3</td>
<td>2,107</td>
</tr>
</tbody>
</table>

The negative charge of total LDL was artificially modified by succinylation of their apoprotein moiety, and the size and electrostatic characteristics of modified LDL were determined as described under Materials and Methods. Data correspond to single determinations and they are representative of two distinct experiments.
starting HDL to $-10.9 \text{ mV}$ in HDL with the highest A-II:A-I ratio.

Comparative study of the effect of the electrostatic charge of plasma HDL subfractions on the activity of the cholesteryl ester transfer protein and of the phospholipid transfer protein.

In order to determine whether variations in lipoprotein electronegativity can influence the interaction of PLTP not only with modified HDL, but also with native plasma HDL subfractions, total native HDL were isolated from human plasma, and they were subsequently fractionated by anion exchange chromatography on a MonoQ column (see Materials and Methods). Collected fractions were pooled in order to constitute five distinct HDL subfractions with increasing electronegative charge. As expected, and in accordance with previous data (13), a good correspondence between the elution delay of the HDL particles from the MonoQ column and their migration distance on agarose gel was observed, with the long-retained particles presenting the highest electronegativity (Table 4). The surface potential was shown to decrease from approximately $-10.5 \text{ mV}$ down to approximately $-12.5 \text{ mV}$ from the first to the last eluting fraction (Table 4). Coincidently, the density of electronegative charges, expressed in $-\text{esu/cm}^2$, increased gradually with the elution delay (Table 4). As previously reported (13), the HDL particles with low electronegative charge tended to be core lipid-rich and contained proportionally more apoA-I than the last-eluting, highly electronegative HDL which presented lower proportions of cholesteryl esters and triglycerides, and elevated A-II:A-I ratios (results not shown).

Subsequently, various HDL subfractions with distinct electronegativity were studied for their ability to serve as substrates in the PLTP-mediated phospholipid transfer reaction. In these experiments, the CETP-mediated cholesteryl ester transfer process was evaluated in parallel by using the same HDL subfractions. To this end, HDL subfractions from the MonoQ column were used as lipoprotein acceptors in experimental mixtures containing either [14C]DPPC-liposomes and PLTP, or [3H]CE-LDL and CETP. As presented in Fig. 4, the val-

### Table 3. Size and charge characteristics of plasma HDL after enrichment with apoA-II

<table>
<thead>
<tr>
<th>Fraction Number</th>
<th>A-II/A-I</th>
<th>Particle Diameter</th>
<th>Agarose Migration</th>
<th>Surface Potential</th>
<th>Density of Surface Charge</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>28.9</td>
<td>8.6</td>
<td>24.6</td>
<td>11.6</td>
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</tr>
<tr>
<td>II</td>
<td>31.5</td>
<td>8.6</td>
<td>24.4</td>
<td>11.5</td>
<td>2,230</td>
</tr>
<tr>
<td>III</td>
<td>41.5</td>
<td>8.6</td>
<td>24.1</td>
<td>11.4</td>
<td>2,208</td>
</tr>
<tr>
<td>IV</td>
<td>50.1</td>
<td>8.8</td>
<td>23.1</td>
<td>11.0</td>
<td>2,118</td>
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<tr>
<td>V</td>
<td>58.9</td>
<td>8.8</td>
<td>22.8</td>
<td>10.9</td>
<td>2,096</td>
</tr>
</tbody>
</table>

Total native HDL (fraction I) were progressively enriched with apoA-II by incubation in the presence of increasing amounts of delipidated HDL (fractions II to V). The size and electrostatic characteristics of apoA-II-enriched HDL were determined as described under Materials and Methods. Data correspond to single determinations and they are representative of two distinct experiments.

### Table 4. Size and charge characteristics of plasma HDL subfractions isolated by anion exchange chromatography

<table>
<thead>
<tr>
<th>Fraction Number</th>
<th>Particle Diameter</th>
<th>Agarose Migration</th>
<th>Surface Potential</th>
<th>Density of Surface Charge</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>9.0</td>
<td>22.8</td>
<td>10.9</td>
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<td>II</td>
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<td>11.5</td>
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<td>IV</td>
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<td>24.8</td>
<td>11.7</td>
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<td>V</td>
<td>9.8</td>
<td>25.0</td>
<td>11.8</td>
<td>2,185</td>
</tr>
</tbody>
</table>

Ultracentrifugally isolated HDL were fractionated by anion exchange chromatography, and the size and electrostatic characteristics of HDL subfractions were determined as described under Materials and Methods. Data correspond to single determinations, and results from three distinct experiments (A, B, and C) are presented.

### Fig. 3. Effect of the surface potential of apoA-II-enriched HDL on the rate of phospholipids transferred from liposomes to HDL in the presence of purified PLTP. Experimental conditions were as described in the legend to Fig. 1. Each point represents the mean ± SD of triplicate determinations. Data are representative of two distinct experiments, and characterization of HDL particles is given in Table 3. Inserted numbers correspond to A-II:A-I + A-II % values.
ues of lipid transfer rates varied with the electronegativity of the HDL subfraction studied. With both lipid transfer systems, the rate of transfer of radiolabeled lipids, i.e. [\(^{14}\)C]DPPC and [\(^{3}\)H]CE, increased progressively as the electronegativity of HDL increased, until a maximal transfer value was reached for a mean surface potential of \(-11.6\) mV in all cases (optimal charge density ranging between \(-2,100\) and \(-2,200\) esu/cm\(^2\)) (see Fig. 4). As the HDL electronegativity kept increasing beyond the optimal surface potential value, progressive decreases in both cholesteryl ester and phospholipid transfer rates were observed. Overall, the general shapes of lipid transfer curves obtained with CETP and PLTP, and presented in Fig. 4, were remarkably similar.

It is noteworthy that measurements of lipid transfer activities and HDL surface potentials were conducted at different pH values (pH 7.4 and pH 8.6, respectively) and with different buffer compositions (TBS buffer and barbital buffer, respectively) (see Materials and Methods). However, PLTP activity did not vary markedly around the physiological pH, and phospholipid transfer rates of \(17.3 \pm 1.6\%\), \(15.8 \pm 0.5\%\), \(16.7 \pm 0.9\%\), \(17.4 \pm 3.6\%\), \(17.7 \pm 1.6\%\), and \(14.5 \pm 0.2\%\) were measured with pH values of 6.5, 7.0, 7.5, 8.0, 8.5, and 9.0, respectively. In the latter experiments, pH was adjusted by using concentrated phosphate-buffered saline solutions of given pH. Moreover, HDL subfractions from the MonoQ column gave similar phospholipid transfer activity curves when measured in the presence of either the TBS, pH 7.4, buffer or the barbital, pH 8.6, buffer, and a maximal transfer value was reached in both cases with the \(-11.6\) mV HDL subfraction (Fig. 5).

**Effect of the electrostatic charge of plasma LDL subfractions on the activity of the phospholipid transfer protein**

Finally, the anion exchange chromatographic procedure previously applied to the fractionation of total

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**Fig. 4.** Comparative effect of the surface potential of plasma HDL subfractions isolated by anion exchange chromatography on the activity of CETP and PLTP. For CETP activity measurements, the rates of cholesteryl esters transferred from LDL to HDL were determined by incubating \([\(^{3}\)H]CE-LDL (2.5 nmol cholesterol) and HDL (10 nmol cholesterol) for 3 h at 37°C in the presence of purified CETP (15 μg) in a final volume of 50 μl. At the end of the incubation, LDL and HDL were separated by ultracentrifugation and CETP activity was calculated as described under Materials and Methods. For PLTP activity determinations, experimental conditions were as described in the legend to Fig. 1. Each point represents the mean ± SD of triplicate determinations. The figure shows results from three independent experiments (A, B, and C), and corresponding fractions are presented in Table 4.
plasma HDL was used to obtain five distinct subfractions from total plasma LDL. Again, the electrophoretic analysis of resulting LDL subfractions in agarose gel revealed a gradual rise in the electronegative charge of lipoprotein particles as the elution delay from the MonoQ column increased (Table 5). Absolute surface potential values, as well as absolute surface charge density values measured with LDL were clearly in a lower range than those measured with HDL subfractions (see Tables 4 and 5). As shown in Fig. 6, PLTP activity increased linearly when LDL electronegativity increased. In contrast to observations made with HDL subfractions, only unimodal transfer curves were obtained within each LDL series (experiments D and E), and maximal lipid transfer rates were constantly measured with the most electronegative LDL subfractions.

### DISCUSSION

Plasma PLTP is susceptible to participate to a number of biological processes by facilitating the transfer of various molecular species, including phospholipids (24, 25), lipopolysaccharides (26), \( \alpha \)-tocopherol (27),...
and unesterified cholesterol (28) among various lipoprotein classes, as well as between lipoproteins and cells. In addition, PLTP has been shown to promote the simultaneous formation of large-sized HDL and preβ HDL through an HDL fusion process (29–32). To date, the mechanism of interaction of PLTP with lipoproteins has not been elucidated, and the main determinants of PLTP activity remain to be identified. Whereas the PLTP-mediated transfer has been reported to be dependent on phospholipid species (33–35), previous studies suggested that alterations in the structure and composition of lipoproteins may also affect spontaneous and facilitated phospholipid transfers (36). Results from the present study demonstrated that the electronegativity of plasma HDL and LDL constitutes an important modulator of the ability of PLTP to transfer phosphatidylcholine, suggesting that the interaction of PLTP with lipoproteins is electrostatic in nature. Similar conclusions were drawn by inducing artificial changes in the charge characteristics of isolated lipoproteins through chemical modification or apolipoprotein substitution, as well as by using native plasma HDL and LDL subfractions with distinct electronegative charges, as naturally occurring in human plasma.

In a first attempt to determine the effect of alterations in the electrostatic charge properties of lipoproteins on PLTP activity, the consequence of gradual succinylation of lysyl residues of apolipoproteins on PLTP-mediated phospholipid transfer rates was evaluated. Although without direct physiological relevance, similar chemical modifications were proven in the past to constitute relevant tools in elucidating the mechanism of interaction of CETP with lipoprotein substrates (11). In the present study, progressive succinylation allowed us to obtain controlled increments in the number of electronegative charges in the lipoprotein surface without inducing alterations in the overall structure and composition of lipoprotein particles. Succinic anhydride-induced changes in the electronegative charge of isolated HDL beyond the mean surface potential value of starting HDL, i.e. from $-11.7$ mV in native HDL down to $-15.7$ mV in the most succinylated particles, was associated with gradual decrease in PLTP-mediated phospholipid transfer rates. Another means to induce controlled changes in the electrostatic charge of HDL in the present study consisted in the progressive replacement of apoA-I by apoA-II in HDL (13). In contrast to succinylation of lysyl residues that was accompanied by gradual increase in HDL electronegativity, the apolipoprotein substitution, without modification of the HDL size and lipid content (16, 23), allowed us to obtain apoA-II-enriched HDL with decreasing electronegativity, probably reflecting the lower electronegative charge of apoA-II as compared with apoA-I (37). As discussed in previous studies (13), native plasma apoA-II-containing HDL were surprisingly of greater electronegativity than apoA-II-poor HDL, sustaining the concept that the electronegative charge of plasma HDL subfractions is mainly dependent on their lipid rather than on their apolipoprotein composition (38). The apolipoprotein substitution experiments also demonstrated that the electrostatic charge of HDL can influence the PLTP-mediated phospholipid transfer reaction. It is noteworthy that progressive decreases in phospholipid transfer rates were observed with both succinylated HDL and apoA-II-enriched HDL series in spite of opposite variations in the electrostatic charge of modified HDL. Based on previous studies that reported a bimodal variation of CETP-mediated cholesteryl ester transfer (11, 13), we made the hypothesis that opposite variations of HDL electrostatic charge on both sides of an optimal value might also account for data obtained in the present study with succinylated HDL and apoA-II-enriched HDL. In fact, assuming that native plasma HDL present an optimal mean surface potential of approximately $-11.6$ mV (13, 18, present study), neither enrichment of HDL with apoA-II, nor succinylation of HDL alone might allow us to obtain progressive alterations of lipoprotein electronegative charge on both sides of the optimal value. In order to circumvent this putative limitation, we took advantage of the lower electronegativity of plasma LDL to use them as starting material in succinylation experiments. The gradual succinylation of isolated LDL allowed us to obtain lipoprotein series with more dispersed surface potential values, ranging between $-4.3$ mV in native LDL and $-14.3$ mV in the most succinylated particles. Under these experimental conditions, we observed a bimodal variation of PLTP activity that was characterized by successive increase and decrease in phospholipid transfer rates along the electronegativity scale.

Although artificial chemical modifications or substitutions in the apolipoprotein moiety of native lipoproteins constituted convenient tools for studying the role of lipoprotein electronegative charges in modulating PLTP activity, they did not mimic the in vivo situation, and in particular they went far beyond the electrostatic variations that are susceptible to occur in plasma. In order to bring more insight into the biological relevance of the role of lipoprotein electronegative charges in determining PLTP activity, native lipoprotein subfractions with distinct electronegative charges were obtained by using anion exchange chromatography.

In contrast to artificial alterations in HDL surface potential induced in the present study of direct modification of HDL apolipoproteins, the determinants of the electrostatic charge of native HDL remain to be clearly identified. Although previous studies suggested that
the electronegativity of HDL would be mainly a function of their lipid content (38), whether it relates to the presence of negatively charged lipid species, such as non-esterified fatty acids (8) or phospholipids (5, 38), or to conformational changes of apolipoprotein A-I (13, 38–40) deserves further investigation. We observed in the present study that differences in the surface potential of plasma HDL, reflecting the heterogeneity of the plasma HDL pool in terms of electrostatic characteristics, significantly modulate PLTP activity. In agreement with the hypothesis resulting from the comparative analysis of data obtained above with succinyolated HDL and apoA-II-enriched HDL, progressive increase in electronegativity of plasma HDL was associated with a bimodal variation of phospholipid transfer values, low or high surface potentials being accompanied by the reduction in phospholipid transfer rates on both sides of a maximal point. Interestingly, previous studies demonstrated that maximal CETP-mediated cholesteryl ester transfer rates correspond also to an optimal electrostatic charge of HDL (11, 13), both excessive and insufficient electrostatic interactions leading to reduced cholesteryl ester transfer activity. In the present study, striking parallelisms between CETP and PLTP transfer activity curves obtained within each HDL series were observed, and the optimal HDL surface potential values required to optimize CETP-mediated and PLTP-mediated transfer reactions were remarkably similar, approximating −11.6 mV in all the experiments involving plasma HDL subfractions (13, present study).

Like HDL, plasma LDL were previously shown to be constituted of an heterogeneous population of subfractions with distinct surface potential, size, and composition (17). Complementary experiments conducted with plasma LDL subfractions obtained by anion exchange chromatography further sustained the role of electronegative charges in modulating PLTP activity. Indeed, gradual increases in LDL electronegativity were associated with parallel, linear rises in PLTP-mediated lipid transfer rates. In contrast to HDL subfractions, a maximal transfer value followed by a gradual decrease in PLTP activity was not observed with plasma LDL subfractions. The differences in the shapes of phospholipid transfer curves obtained with HDL and LDL series might relate to the fact that the absolute surface potential values measured in plasma LDL were clearly in a lower range than those measured in plasma HDL (−3.5/−5.0 mV in plasma LDL versus −10.5/−12.5 mV in plasma HDL). Although it was also possible to obtain a bimodal transfer curve with highly electronegative LDL that was artificially generated by succinylation, the optimal surface potential value seemed to differ from that found with HDL, suggesting that not only the mean electrostatic charge, but also the size and the composition of lipoprotein particles can modulate their ability to interact with PLTP.

The observations of the present report suggest that the interaction of CETP and PLTP with lipoproteins can rely on similar mechanisms, involving positively charged groups of lipid transfer proteins and negative charges of the lipoprotein surface. Whereas a triplet of lysine residues in positions 377–379 (10), as well as the carboxyl-terminal sequence (41, 42) of CETP were successively described as putative lipoprotein-binding sites in earlier studies, recent mutagenesis experiments clearly demonstrated that two distinct basic aminoacid residues, i.e. Lys233 and Arg259, play a determinant role in CETP activity (43). Concordant observations were made by using specific monoclonal antibodies (44). It results from these studies that the binding of positive charges of Lys233 and Arg259 to negatively charged groups at the lipoprotein surface could constitute one key step of the CETP-mediated neutral lipid transfer reaction. Interestingly, multiple sequence alignment among members of the LT/LBP gene family revealed that positively charged, basic aminoacid residues are remarkably conserved in similar positions (43). More precisely, two arginine residues of PLTP, i.e. Arg218 and Arg245, correspond to Lys233 and Arg259 of CETP (3, 43), suggesting that these homologous amino acids may exert similar functions. In spite of the net negative charge of PLTP at the working pH (pI of PLTP, 5.0) (25), the elevated, 12.48 pK of the guanidinium group of the arginine residue indicates that it is positively charged around the physiological pH, and similar phospholipid transfer values were measured in the 6.5–9.0 pH range. Therefore, findings of the present report raise the hypothesis of a putative role for Arg218 and Arg245 residues of PLTP in mediating its electrostatic interaction with negative charges at the lipoprotein surface.

In conclusion, the electrostatic charge of modified and native lipoproteins was shown to influence the phosphatidylcholine transfer activity of PLTP. As similar charge-dependent variations were observed when evaluating the cholesteryl ester transfer activity of CETP and the phospholipid transfer activity of PLTP with the same lipoprotein subfractions, it is suggested that the interaction of the two plasma lipid transfer proteins with lipoproteins might rely on similar molecular mechanisms. As previously observed with CETP, the electrostatic interaction might constitute a rate-limiting step of the PLTP-mediated lipid transfer reaction, with either insufficient or excessive lipoprotein electronegativity causing decreased lipid transfer rates. However, it is noteworthy that differences might exist in the ability of PLTP and CETP to interact with various lipoprotein substrates. For instance, PLTP has been shown to be mainly associated with the plasma HDL2...
fraction whereas CETP is preferentially associated with HDL3 (45), and only PLTP, but not CETP, was shown to promote the transfer of phosphatidylcholine from liposomes (20). These observations indicate, therefore, that the overall electrostatic charge of lipoproteins does not constitute the sole rate-limiting factor of the lipid transfer reactions, and they suggest that other factors, such as curvature or fluidity of lipoproteins, might affect differently the interaction of CETP and PLTP.

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