Molecular Mechanism of the Blockade of Plasma Cholesteryl Ester Transfer Protein by Its Physiological Inhibitor Apolipoprotein CI*

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Genetically engineered mice demonstrated that apolipoprotein (apo) CI is a potent, physiological inhibitor of plasma cholesteryl ester transfer protein (CETP) activity. The goal of this study was to determine the molecular mechanism of the apoCI-mediated blockade of CETP activity. Kinetic analyses revealed that the inhibitory property of apoCI is independent of the amount of active CETP, but it is tightly dependent on the amount of high density lipoproteins (HDL) in the incubation mixtures. The electrostatic charge of HDL, i.e. the main carrier of apoCI in human plasma, is gradually modified with increasing amounts of apoCI, and the neutralization of apoCI lysine residues by acetylation produces a marked reduction in its inhibitory potential. The inhibitory property of full-length apoCI is shared by its C-terminal α-helix with significant electrostatic properties, whereas its N-terminal α-helix with no CETP inhibitory property has no effect on HDL electronegativity. Finally, binding experiments demonstrated that apoCI and to a lower extent its C-terminal α-helix are able to disrupt CETP-lipoprotein complexes in a concentration-dependent manner. It was concluded that the inhibition of CETP activity by apoCI is in direct link with its specific electrostatic properties, and the apoCI-mediated reduction in the binding properties of lipoproteins results in weaker CETP-HDL interactions and fewer cholesteryl ester transfers.

The cholesteryl ester transfer protein (CETP) mediates the exchange of neutral lipids, i.e. cholesteryl esters and triglycerides between plasma lipoproteins (1, 2). Through its action, CETP can influence the atherogenicity of the lipoprotein profile (2–4), and recent studies (5–7) support a potential interest in inhibiting CETP activity in vivo by means of either anti-CETP immunotherapy, antisense oligonucleotides, or specific pharmacological inhibitors. In particular, the latest pharmacological interventions with small anti-CETP molecules in human populations demonstrated that CETP inhibition markedly increases HDL cholesterol levels and also decreases low density lipoprotein (LDL) cholesterol levels (8–10). Most interestingly, previous studies (11–13) in human populations are consistent with the association of high CETP with an increase in coronary heart disease, in particular in subjects with elevated triglycerides.

Besides interventional studies with exogenous compounds, a number of studies (14–19) indicated that plasma CETP activity can be modulated by endogenous factors, among them the apolipoprotein components of circulating lipoproteins. Recently, apolipoprotein CI, i.e. a 6.6-kDa HDL apolipoprotein, was identified as a potent CETP inhibitor (20). In contrast to other putative apolipoprotein candidates that were identified only through in vitro experiments, the ability of apoCI to decrease specific CETP activity was documented in vivo through studies in CETP−/apoCI-KO and CETP+HuapoCITg (transgenic mouse to both human CETP and human apolipoprotein CI) mice (21, 22). Although it was concluded that apoCI constitutes the major physiological inhibitor of CETP in the plasma compartment, the molecular mechanism of the blockade of the cholesteryl ester transfer reaction by apoCI remained to be identified. The CETP-mediated lipid transfer reaction is a complex process, with at least two rate-limiting steps. In the first step, CETP binds to lipoproteins through electrostatic interactions with negative charges localized at the lipoprotein surface (23–25). In the second step, and after a conformational change of CETP, one neutral lipid molecule binds to an hydrophobic site in the C terminus of the protein prior to be transferred to the lipoprotein acceptor (26, 27). In concordance with the two steps of the CETP-mediated transfer reaction, at least two distinct ways of CETP blockade were reported in previous studies. First, CETP inhibition may result from either insufficient or excessive binding of CETP at the lipoprotein surface in step 1 of the lipid transfer process, and both weak CETP-lipoprotein interaction (25, 28) and strong, sometimes irreversible, CETP-lipoprotein association (10, 25, 27) results in significant inhibition of the lipid transfer reaction. Second, CETP inhibition may result from the blockade of the neutral lipid binding site in step 2 of the lipid transfer process, resulting in an abnormal production of irreversibly associated CETP-lipoprotein complexes in this case (8, 27, 29, 30).

In the present study, the effect of apoCI on the lipid transfer process was determined in a way concordant in vitro observations indicate that the inhibitory property of apoCI is in a direct link with its electrostatic charge properties and its ability to produce significant changes in CETP-lipoprotein interactions.
MATERIALS AND METHODS

Plasma Samples—Fresh citrated plasmas from normolipidemic subjects were provided by the Etablissement Français du Sang (Hôpital du Bocage, Dijon, France).

Isolation of HDL Particles—Total HDL were ultracentrifugally isolated from normolipidemic human plasmas as the 1.070 < d < 1.210 g/ml fraction, with one 24-h, 45,000 rpm spin at the lowest density and one 24-h, 50,000 rpm spin at the highest density in a 70 Ti rotor in an L90-K ultracentrifuge (Beckman Instruments). HDL2 and HDL3 were isolated as the 1.070 < d < 1.125 g/ml and the 1.125 < d < 1.210 g/ml fractions, respectively. Isoelectrofocusing of HDL2 and HDL3 was conducted in an NVT-90 rotor in an L90-K ultracentrifuge (Beckman Instruments), with a 2.5-h, 90,000 rpm spin at density 1.070 g/ml, a 3-h, 90,000 rpm spin at density 1.125 g/ml, and a 3.5-h, 90,000 rpm spin at density 1.210 g/ml. Densities were adjusted by the addition of solid KBr. Isolated HDL, HDL2, and HDL3 were dialyzed overnight against 10 mmol/liter Tris-buffered saline, pH 7.4, with 3 mmol/liter Na2SO4 (TBS buffer). CETP was purified from human plasma by using a sequential chromatography procedure as described previously, and CETP preparation was deprived of both lecithin/cholesterol acyltransferase and phospholipid transfer protein activities (32, 33).

Apolipoprotein CI Acetylation—Apolipoprotein CI was acetylated as described previously (34). Briefly, 100 µg of purified apoCI was incubated for 1 h at room temperature with a saturated solution of sodium acetate at the presence of increasing amounts of acetic anhydride (range, 0–21 µmol).

Anti-apoCI Immunoaffinity Chromatography—ApoCI was removed from total human HDL by passage through an anti-apoCI immunoaffinity column as described previously (20). ApoCI-poor HDL that did not bind to the immunosorbent column were washed off with TBS buffer, and their ability to exchange cholesteryl esters was compared with total HDL.

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Isoelectrofocusing Analysis—Native apoCI (5 µg) and apoCI treated with acetic anhydride (5 µg) or protein pl standards (two-dimensional SDS-PAGE; Bio-Rad) (10 µl) were diluted in 300 µl of hydration buffer (8 mol/liter urea, 4% CHAPS, 20 mmol/liter dithiothreitol, 0.2% Bio-Lyte 3–10). After an overnight hydration of 17-cm-long ReadyStrip, pH 3–10 (Bio-Rad), at 50 V in a Protein IEF cell (Bio-Rad), isoelectrofocalization was conducted for 40 kV-h. Strips were stained with Coomassie Brilliant Blue G-250 and analyzed on a GS-800 calibrated densitometer (Bio-Rad).

Matrix-assisted Laser Desorption Ionization-Time-of-Flight (MALDI-TOF) Mass Spectrometry of Native and Acetylated ApoCI—Prior to mass spectrometry analysis, native and acetylated apoCI were desalted on ZipTip microcolumns (ZipTip µC18, Millipore) as described by the manufacturer. Briefly, 1 µg of protein in 10 µl was acidified with trifluoroacetic acid (final concentration, 0.1% trifluoroacetic acid). ZipTips were pre-wetted with 10 µl of 50% acetonitrile in MilliQ grade water and equilibrated with 10 µl of 0.1% trifluoroacetic acid. Bound proteins were washed twice with 10 µl of 0.1% trifluoroacetic acid, and they were finally eluted with 4 µl of 0.1% trifluoroacetic acid, 50% acetonitrile in MilliQ grade water. One microliter of eluted protein was mixed with 4 µl of a saturated solution of α-cyano-4-hydroxyphenylacetamide (Bruker Daltonique S.A., Wisseembourg, France) in a 0.1% acetonitrile/trifluoroacetic acid (1:2, v/v) solution. Finally, 1 µl of the mixture was spotted on a MTP348 ground steel target plate (Bruker Daltonique S.A.).

The MALDI-TOF mass spectrometric measurements were performed on a Ultraflex II TOF/TOF spectrometer (Bruker Daltonique S.A.) in positive 25-kV linear mode. Insulin (Mw = 5734.56), ubiquitin I (Mw = 8856.89), cytochrome c (Mw = 12361.09), and myoglobin (Mw = 16952.55) were used as external calibration standards (protein calibration standard I, Bruker Daltonique S.A.).

Measurement of Cholesteryl Ester Transfer Activity—CETP activity was determined in microplates by a fluorescent method using donor liposomes enriched with nitrobenzoxadiazol (NBD)-labeled cholesteryl esters (phospholipid/cholesterol/NBD-cholesterol ester molar ratio, 1:1:1) (Roar Biomedical). For measurements of cholesteryl ester transfer activity with isolated CETP, donor liposomes (phospholipids, 5 µmol/liter) and purified CETP (range, 2–16 µg) were incubated in the presence of HDL (range, 0.5–4.0 µmol/liter) or LDL (7 µg of cholesterol), as indicated. For measurement of cholesteryl ester transfer activity in total plasma, incubation media contained donor liposomes (phospholipids, 5 µmol/liter) and 10 µl of plasma. Final volumes were adjusted to 250 µl with TBS (unless specified), and incubations were conducted in triplicate for 3 h at 37 °C in a Victor2 1420 multilabel counter (PerkinElmer Life Sciences). The CETP-mediated transfer of NBD-cholesterol esters from self-quenched donors to acceptor lipoproteins was monitored by the increase in fluorescence intensity (excitation, 465 nm; emission, 535 nm), and results were expressed in fluorescence arbitrary units after deduction of blank values that were obtained with control mixtures without CETP.

SDS-PAGE—HDL apolipoproteins were delipidated with ethanol/ether (3:2), diluted in the sample buffer, and applied on 4–12% NuPAGE BisTris Novex SDS-polyacrylamide gels as recommended by the manufacturer (Invitrogen). Proteins were stained with Coomassie Brilliant Blue G-250, and the apparent molecular weights of individual protein bands were determined by reference to protein standards (Mark12, Invitrogen).

Agarose Gel Electrophoresis—The electrophoretic mobility (U in the following equations) of HDL was determined by electrophoresis on 0.5% agarose gels (Paragon Lipo kit, Beckman Instruments) according to the method of Sparks and Phillips (35). Briefly, gels were cast in a Sebasto K20 system, and electrophoresis was performed for 45 min at 100 V in barbital buffer, pH 8.6. After electrophoresis, the gels were successively fixed for 5 min in an ethanol/acidic acid/water 60:10:30 solution, dried, stained for 5 min in a 0.07% solution of Sudan Black B in ethanol/water 55:45, and destained for 10 min with a solution of ethanol/water 45:55. In parallel, gel portions containing purified bovine serum albumin, which was used as an internal standard, were stained with a 0.8 g/liter solution of Coomassie Brilliant Blue G-250 in a methanol/acidic acid/water 10:1:10 solution and destained in a solution of methanol/acidic acid/water 2:3:40. Mean migration distances were obtained by using the GelDoc analysis software (Bio-Rad).

Calculation of Electrophoretic Mobility and Surface Potential of HDL Particles—Surface charges of HDL were determined as described previously (35). Briefly, electrophoretic mobilities (U) were calculated by dividing the electrophoretic velocity (mean migration distance (mm) per time in seconds) by the electrophoretic potential (voltage per gel distance in centimeters). To correct the pl-dependent retardation effects, the Equation 1 was applied (35).

\[
U \text{ corrected } = (U \text{ agarose } - 0.136)/1.211
\] (Eq. 1)

The surface potentials of HDL were calculated by using the Henry’s Equation 2 (see Ref. 35).
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\[ S = U \times \frac{6\pi n}{D} \]  

(Eq. 2)

where \( n \) is the coefficient of viscosity (0.0089 poise), and \( D \) is the solvent dielectric constant.

Formation of CETP-Lipoprotein Complexes—Ultracentrifugally isolated HDL were covalently bound to CNBr-activated Sepharose 4B (Amersham Biosciences) at a ratio of 10 mg of HDL proteins g of gel as recommended by the manufacturer. The HDL-Sepharose phase was resuspended in PBS containing 3 mmol/liter NaN₃. Each incubation as recommended by the manufacturer. The HDL-Sepharose phase was resuspended in PBS containing 3 mmol/liter NaN₃. Each incubation was conducted for 3 h at 37 °C in the absence or in the presence of purified apoCI (concentration, 0.5 μmol/liter). Bottom panel, percentage of CETP inhibition (vertical bars A–D) was calculated by comparing the initial transfer rate in the presence of apoCI to the initial transfer rate with no apoCI added (A–D in top panel). Similar blank values were obtained whether donor liposomes were incubated with or without apoCI alone. Initial transfer rates were determined from the linear, initial portion of the time course curves. Plotted values and vertical bars are the mean ± S.D. of three determinations.

\[ \text{CETP inhibition (\%)} = \frac{U_{\text{control}} - U_{\text{sample}}}{U_{\text{control}}} \times 100 \]

where \( U \) is the transfer activity (fluorescence arbitrary units).

Blots were blocked for 30 min at 37 °C in 3% low fat dried milk in Tris-buffered saline containing 0.1% Tween, and they were washed in Tris-buffered saline/Tween. CETP was revealed by successive incubations with TP1 anti-CETP antibodies (Heart Institute, Ottawa, Canada) and horseradish peroxidase-coupled secondary antibodies as described previously (36). Blots were finally developed with an ECL kit (Amersham Biosciences). Band intensities were measured by using the GelDoc analysis software (Bio-Rad). The amount of CETP in each supernatant was determined by comparison with a calibration curve that was obtained with known amounts of purified CETP that were applied to the membrane together with the samples.

**Lipid and Protein Analyses**—All assays were performed on a Victor₂ 1420 multilabel counter (PerkinElmer Life Sciences). Total cholesterol was measured by the enzymatic method using the Cholesterol 100 reagent (ABX Diagnostics). Phospholipids and triglycerides were determined by enzymatic methods, as described previously (21). Protein concentration was measured by using the bichinonic acid reagent (Pierce).

**Statistical Analyses**—Mann-Whitney U test was used to determine the statistical significance between data means.

**RESULTS**

Comparative Effects of Increasing Levels of Human HDL Versus Human CETP on the ApoCI-mediated Inhibition of the Cholesterol Ester Transfer Reaction—When the amount of fluorescent liposome donors and HDL acceptors were kept constant in the CETP activity assay, the
chol esteryl ester transfer rate measured over a 3-h incubation period increased gradually with the amount of purified CETP added (from 2 μg in Fig. 1A to 16 μg in Fig. 1D). In all cases, and in accordance with previous *in vitro* and *in vivo* studies (20, 21), the addition of apoCII was accompanied by a marked inhibition of the lipid transfer reaction. The inhibitory potential of a given amount of apoCII appeared to be independent of the amount of CETP added, in all cases with a constant ~70% reduction in cholesteryl ester transfer rate as compared with control mixtures with no apoCII added. Conversely, the inhibitory potential of purified human apoCII was markedly affected by the amount of HDL acceptors that were added to the incubation mixture, with CETP kept constant. In the latter case, the capacity of human apoCII to block the lipid transfer process, approximating 80% of inhibition in incubation mixtures with the lowest HDL levels, was completely abolished with the highest HDL concentration studied (Fig. 2). CETP inhibition by HDL is the consequence of a direct and specific property of apoCII, because apoCII-poor HDL displayed a much weaker ability to block the lipid transfer reaction in the HDL concentration range studied (Fig. 3). The apolipoprotein profiles of apoCII-poor HDL as prepared by anti-apoCII immunoaffinity chromatography and of native HDL differed only by their apoCII content (Fig. 3). Percent composition of the lipid moiety did not differ significantly between total and apoCII-poor HDL from three distinct preparations (total cholesterol %, 35.9 ± 2.3 versus 31.9 ± 1.6, respectively; triglyceride %, 8.2 ± 0.5 versus 9.9 ± 1.1, respectively; and phospholipid %, 55.9 ± 2.4 versus 58.1 ± 0.9, respectively). Overall, the results showed that the capacity of apoCII to block the lipid transfer reaction was independent of the amount of active CETP, but it was tightly dependent on the amount of HDL acceptors in the incubation mixtures.

**Effect of Purified ApoCII on the Electrostatic Charge Properties of HDL**—As illustrated in Fig. 4, apoCII produced a significant change in the surface charge properties of plasma HDL. Mean surface potential of native HDL increased gradually from −11.7 to −8.8 mV as the apoCII to HDL molar ratio increased from 0 to 6.4 (Fig. 4). Complementary experiments demonstrated that apoCII is able to modify the charge characteristics of both isolated HDL2 and HDL3, however, with a greater impact on HDL3 than on HDL2 at the apoCII to HDL molar ratio of 1.6 and 3.2 (Fig. 5).

**Loss of CETP Inhibitory Property of Acetylated ApoCII**—To bring more insight into the role of basic, positively charged residues of apoCII in mediating its lipid transfer inhibitory property, purified human apoCII was incubated with increasing concentrations of acetic anhydride, *i.e.* a neutralizing agent of positively charged groups of lysine residues (34). As checked by MALDI-TOF analysis, incubation of human apoCII in the presence of 0.5 μmol of acetic anhydride produced 42-Da increments in

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the molecular mass of the [M – H]^+ ion corresponding to the acetylation of 1–3 lysine residues (Fig. 6B). In the presence of 21 μmol of acetic anhydride, and as compared with the apoCI 6632 [M – H]^+ ion (Fig. 6A), the main 7052 [M – H]^+ ion corresponded to 10 42-Da increments (i.e. nine lysine modifications plus one N terminus modification) (Fig. 6C). As illustrated in Fig. 7, acetylation of apoCI in the presence of acetic anhydride led to the emergence of new isoforms as observed by isoelectrofocusing, with a gradual shift of apparent pI from 8.3 with native apoCI down to 3.2 with apoCI treated with the highest amount of acetic anhydride (Fig. 7). In the meantime, CETP activity rose from 10 (with native apoCI) to 77% (with acetylated apoCI) of control transfer values measured in the absence of apoCI, indicating that the inhibitory potential of apoCI is markedly reduced as a result of the neutralization of its lysine residues (Fig. 7).

Despite a significant reduction of apoCI chromogenicity after acetylation (Fig. 8A), acetylated apoCI was found to readily associate with HDL, and as for native apoCI, association with HDL did not induce profound changes in the apolipoprotein composition of the particles as shown by polyacrylamide gradient gel electrophoresis (Fig. 8B). Most strikingly, the binding of acetylated apoCI to HDL, unlike the binding of native apoCI (Fig. 4), did not produce a marked reduction in HDL electronegativity, even with an opposite tendency to shift toward lower surface potential values in the presence of increasing levels of acetylated apoCI (Fig. 9).

Differential Effects of Full-length or Fragmented ApoCI on CETP Activity and HDL Electronegativity—Two apolipoprotein CI fragments, i.e. fragment 34–54 and fragment 4–25 corresponding to the two distinct α-helices of human apoCI, were synthesized (Fig. 10A). Their ability to inhibit CETP activity was monitored either in reconstituted mixtures containing isolated lipoproteins and purified CETP (Fig. 10B) or in total human plasma (Fig. 10C). Again, and with the two experimental systems, full-length human apoCI was able to block the lipid transfer...
ApoCI is a direct consequence of its unique electrostatic properties, which lead to alteration of the HDL-CETP interactions. In the present study, as conducted through gradual changes in the level of apoCI concentration, we observed that apoCI can dissociate CETP-mediated lipid transfer complexes in a concentration-dependent manner, i.e., apoCI can bind to CETP at various concentrations and inhibit CETP activity. These observations indicate that apoCI can act as a potent and physiological inhibitor of CETP activity. These findings suggest that apoCI is a key player in the regulation of CETP-mediated lipid transfer and its role in the prevention and treatment of atherosclerosis.

**DISCUSSION**

Over the last decade, CETP has emerged as a new target in the prevention and treatment of atherosclerosis, and recent human studies with specific pharmacological inhibitors supported the potent effect of CETP inhibition on the plasma lipoprotein profile, including both a significant rise in HDL cholesterol and a significant decrease in LDL cholesterol (8-10). Besides interventional studies with exogenous compounds, plasma is known to contain a specific lipid transfer inhibitory protein, i.e., apolipoprotein CI (20-22). ApoCI in HDL can suppress the CETP-mediated lipid transfer process in a concentration-dependent manner, and in vivo studies in apoCI-knockout mice expressing human CETP supported the ability of apoCI to act as a potent and physiological inhibitor of CETP activity. These observations indicate that the effect of apoCI on CETP activity is a direct consequence of its unique electrostatic properties that lead to alteration of the HDL-CETP interactions.
either purified CETP or HDL, indicated that the inhibitory potential of apoCI is dependent on the amount of HDL but not on the amount of CETP added. These observations support the hypothesis that apoCI may inhibit CETP through its ability to modify the HDL substrate, rather than through a specific blockade of the CETP molecule. This observation led us to consider in greater detail the specific molecular properties of apoCI and, in particular, the physicochemical changes it induces when combined with HDL, i.e., its main carrier in plasma (39). It must be emphasized at this stage that apoCI, the smallest molecule among the apolipoprotein family, is a highly basic apolipoprotein molecule with a cluster of lysine residues in its C-terminal region (31). The peculiar electrostatic properties of apoCI were shown in vitro to be able to produce a significant change in HDL electronegativity, which is recognized today as a leading parameter determining both the strength of CETP-HDL interactions and the velocity of CETP-mediated lipid transfers (23–25, 40). In accordance with earlier transfer studies with native or chemically modified HDL subpopulations (25, 40), alteration in the mean surface potential of apoCI-enriched HDL corresponded to a shift from optimal values in native HDL particles (mean surface potential of /H1100211.7 mV) to inappropriate values in apoCI-enriched particles. The resulting tremendous changes in both CETP interaction and neutral lipid exchange velocity are in agreement with previous data that indicated that lipid exchange between HDL subpopulations becomes minimal when surface potential is above −11.0 mV (25). In direct link with the implication of positively charged amino acid residues of apoCI in mediating its inhibitory effect, the inhibitory potential of apoCI was lost by acetylation of lysine residues.

Although the CETP inhibitory potential of apoCI is biologically sig-

FIGURE 10. Effect of apoCI fragments on CETP activity. The sequences of synthetic apoCI fragments corresponding to the putative amphipathic α-helices of native apoCI are shaded gray (A). Cholesteryl ester transfer activity was determined as the initial rate of transfer of fluorescent NBD-cholesteryl esters from liposome donors to LDL acceptors in the presence of purified CETP (B) or to plasma lipoproteins in total human plasma samples (C). Incubations were conducted for 3 h at 37 °C in the presence of increasing amounts of purified apoCI or synthetic apoCI fragments (concentration range, 0–5 μmol/liter). Blank values were obtained from homologous incubated mixtures without CETP. Each point represents the mean ± S.D. of triplicate determinations. Asterisks show significant differences compared with initial transfer values with no addition, p < 0.05; Mann-Whitney test.

FIGURE 11. Effect of apoCI fragments on the electronegative charge of HDL. The charge of HDL that were incubated in the presence of increasing amounts of purified apoCI (see “Materials and Methods”) or synthetic apoCI fragments was determined as described in Fig. 4. Results are expressed as the change in electrostatic charge as compared with HDL incubated in the absence of apoCI. Each point is the mean ± S.D. of triplicate determinations with three different HDL preparations. Asterisks show significant differences compared with the initial value with no addition, p < 0.05; Mann-Whitney test.

either purified CETP or HDL, indicated that the inhibitory potential of apoCI is dependent on the amount of HDL but not on the amount of CETP added. These observations support the hypothesis that apoCI may inhibit CETP through its ability to modify the HDL substrate,
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significant, with more pronounced CETP-mediated changes in plasma lipoproteins in CETP-Tg/apoCI-KO mice versus CETP-Tg mice (21). ApoCI overexpression in apoCI transgenic mice increases the circulating levels of CETP (22). This is explained by an indirect, stimulatory effect on the CETP gene in response to marked hyperlipidemia that is due to the inhibition by the elevated levels of apoCI of both lipoprotein lipase activity and binding of VLDL to their receptors (41–45). It results that overexpression of native apoCI does not represent a suitable method for decreasing total cholesteryl ester transfer activity in vivo despite a beneficial impact on the specific activity of CETP. In contrast to initial studies with full-length native apoCI, the structure-function analysis conducted in the present study revealed that a C-terminal fragment of apoCI with no hyperlipidemic potential (43) is sufficient to exert a significant inhibitory effect on CETP. Again, the ability of the C-terminal fragment of apoCI to block the lipid transfer reaction paralleled concomitant changes in the electrostatic charge of HDL. It is noteworthy that the N-terminal α-helix of apoCI with no CETP inhibitory properties had no effect on HDL electronegativity, indicating that differences in the effectiveness of C-terminal and N-terminal apoCI fragments reflect at least in part differences in their intrinsic physicochemical properties. Most interestingly, and as observed with native apoCI, only the C-terminal fragment and not the N-terminal fragment was able to undergo self-association in aqueous buffer and in the concentration range used in the present studies (results not shown). The biological relevance of a specific C-terminal apoCI fragment both in vivo and in vivo studies.

Overall, the observations of the present study converge on a role of apoCI in reducing concomitantly the electronegativity of HDL and the binding of CETP to the lipoproteins. The latter point was confirmed by the redistribution of CETP toward a free, nonlipoprotein-associated pool in the presence of increasing concentrations of positively charged apoCI. Most interestingly, the C-terminal fragment of apoCI that was able to reduce the electronegativity of HDL could also disrupt the binding of CETP to lipoproteins, however, with a weaker efficiency than native apoCI. The electrochemical charge of lipoproteins is a leading parameter in determining the CETP-lipoprotein interaction in the first step of the CETP-mediated lipid transfer process. In the studies of Sammet and Tall (24) with lipolyzed VLDL and HDL, conditions that were shown to increase the transfer of HDL cholesteryl esters were proven to favor the binding of CETP to lipoproteins. In addition, Nishida et al. (23) reported stronger affinity of CETP for succinyllated or acetylated lipoproteins.

The molecular mechanism of CETP inhibition by apoCI and its C-terminal fragment seems to differ from the molecular mechanisms of the two pharmacological inhibitors of CETP that have been studied so far in human populations. On the one hand, the JTT-705 compound and its analogs were shown to inactivate a free sulfhydryl group in the hydrophobic site on CETP, thus resulting in an irreversible inhibition of the binding and transfer of neutral lipids (7, 8, 29). On the other hand, torcetrapib was shown to induce a shift of CETP from a free form to an HDL-bound, nonproductive complex (9, 10). Most interestingly, earlier studies demonstrated that similar excessive binding of CETP to lipoproteins accompanies the inhibition of the CETP-mediated lipid transfer reaction by specific anti-CETP monoclonal antibodies (27). In other words, inhibition of neutral lipid binding to CETP and enhanced association of CETP with lipoproteins might constitute two distinct ways to CETP inhibition in vivo. In addition, the present study indicates that insufficient binding of CETP to apoCI-containing HDL may also constitute another means of CETP blockade. The latter mechanism is compatible with the proposed hypothesis of the existence of an optimal interaction of CETP with native plasma HDL, with both excessive and insufficient CETP-HDL interactions resulting in lower cholesteryl ester transfer rates (25, 40).

In conclusion, the inhibition of CETP activity by apoCI is dependent at least in part to its peculiar electrostatic properties that are able to shift HDL toward one profile of lower electronegativity. Based on earlier studies, relatively subtle alterations in the composition of the lipoprotein surface can influence, sometimes dramatically, the binding of CETP to lipoproteins and thereby the lipid transfer rate. Thus, it is highly probable that controlled, apoCI-mediated alteration of the binding of CETP to lipoproteins may result in fewer cholesteryl ester transfers in human plasma.

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