Remodeling of apolipoprotein E-containing spherical reconstituted high density lipoproteins by phospholipid transfer protein

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Abstract **Phospholipid transfer protein (PLTP) transfers phospholipids between HDL and other lipoproteins in plasma. It also remodels spherical, apolipoprotein A-I (apoA-I)-containing HDL into large and small particles in a process involving the dissociation of lipid-free/lipid-poor apoA-I. ApoE is another apolipoprotein that is mostly associated with large, spherical HDL that do not contain apoA-I. Three isoforms of apoE have been identified in human plasma: apoE2, apoE3, and apoE4. This study investigates the remodeling of spherical apoE-containing HDL by PLTP and the ability of PLTP to transfer phospholipids between apoE-containing HDL and phospholipid vesicles. Spherical reconstituted high density lipoproteins (rHDL) containing apoA-I [(A-I)rHDL], apoE2 [(E2)rHDL], apoE3 [(E3)rHDL], or apoE4 [(E4)rHDL] as the sole apolipoprotein were prepared by incubating discoidal rHDL with low density lipoproteins and lecithin:cholesterol acyltransferase. PLTP remodeled the spherical, apoE-containing rHDL into large and small particles without the dissociation of apoE. The PLTP-mediated remodeling of apoE-containing rHDL was more extensive than that of (A-I)rHDL. PLTP transferred phospholipids from small unilamellar vesicles to apoE-containing rHDL in an isoform-dependent manner, but at a rate slower than that for spherical (A-I)rHDL. It is concluded that apoE enhances the capacity of PLTP to remodel HDL but reduces the ability of HDL to participate in PLTP-mediated phospholipid transfers.**—Settasatian, N., P. J. Barter, and K-A. Rye. **Remodeling of apolipoprotein E-containing spherical reconstituted high density lipoproteins by phospholipid transfer protein.** ***J. Lipid Res.*** **2008.** 49: **115–126.**

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Apolipoprotein E (apoE) is a 299 residue, 34.2 kDa protein that consists of a 22 kDa N-terminal receptor binding domain and a 10 kDa C-terminal lipid binding domain (1). The evidence that apoE protects against atherosclerosis is compelling (1). Three isoforms of apoE with cysteine/arginine interchanges at positions 112 and 158 have been identified in human plasma (2, 3). In apoE2, both of these positions contain cysteine residues, whereas apoE4 has arginine residues. ApoE3, by contrast has a cysteine residue at position 112 and arginine at position 158.

Approximately 70% of the apoE in normolipidemic plasma is associated with large, spherical HDL particles that do not contain apoA-I or apoA-II (4–8). ApoE is also the most abundant apolipoprotein in the brain, and it is present in cerebrospinal fluid as a component of large, spherical HDL (9, 10). As is the case with the apoE-containing HDL in plasma, most of these particles do not contain apoA-I (11–14). Evidence from in vitro studies suggests that apoE-containing HDL play a key role in lipid transport and are important for maintaining cholesterol homeostasis in the brain (15).

Phospholipid transfer protein (PLTP) is a 476 residue glycoprotein with an apparent molecular mass of 81 kDa. It is synthesized in a wide range of tissues, including the liver, lung, kidney, and macrophages (16, 17). The PLTP in human plasma transfers phospholipids between HDL and other lipoproteins as well as between different HDL particles (18, 19). PLTP exists in an active as well as an inactive form (20, 21). Although some investigators have reported that the high-activity form of PLTP is associated with apoE-containing HDL (20, 22), others have not found this to be the case (23).

PLTP is also synthesized in the brain in glial cells and neurons, and high levels of PLTP activity have been reported in cerebrospinal fluid (24). The finding that PLTP

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Abbreviations: apoE, apolipoprotein E; CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; DPPC, 1,2-dipalmitoyl L-3-phosphatidylcholine; PLTP, phospholipid transfer protein; rHDL, reconstituted high density lipoprotein; UC, unesterified cholesterol.

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increases the secretion of apoE from astrocytes indicates that it may regulate apoE levels in the brain (25). There is also evidence to suggest that apoE regulates PLTP activity in plasma, especially in subjects with type 2 diabetes (26).

PLTP contributes to the heterogeneity of plasma HDL. Work from this and other laboratories has shown that PLTP remodels apoA-I-containing HDL into large and small particles by a process that involves particle fusion and the dissociation of lipid-free or lipid-poor apoA-I (27–29).

The aims of the present study were to determine the following: *i*) whether PLTP also remodels apoE-containing HDL; *ii*) the mechanism of the remodeling; *iii*) whether the remodeling is regulated by apoE isoforms; and *iv*) whether apoE-containing HDL participate in PLTP-mediated phospholipid transfers.

To achieve this goal, it was necessary to acquire substantial amounts of HDL containing apoE2, apoE3, or apoE4 as the sole apolipoprotein. This was achieved by generating well-characterized preparations of spherical reconstituted high density lipoproteins (rHDL) containing recombinant apoE2 [(E2)rHDL], apoE3 [(E3)rHDL], or apoE4 [(E4)rHDL] as the only apolipoprotein (30, 31). Previous studies from this laboratory have indicated that the interactions of these rHDL preparations with plasma factors are indistinguishable from that of plasma HDL (32–34).

The results show that PLTP remodels apoE-containing rHDL into large and small particles by a process that involves multiple particle fusions and rearrangements and that these events do not cause lipid-free or lipid-poor apoE to dissociate from the particles. Evidence that apoE decreases the rate at which PLTP transfers phospholipids from small unilamellar vesicles to rHDL is also presented.

EXPERIMENTAL PROCEDURES

Isolation of apoA-I

HDL were isolated from pooled, autologously donated samples of human plasma (Gribbles Pathology, Adelaide, Australia) and delipidated as described (35). The resulting apoHDL were subjected to anion-exchange chromatography on a column of Q Sepharose Fast Flow (GE Healthcare, Uppsala, Sweden) attached to an ÄKTA FPLC system (GE Healthcare) (36, 37). The resulting apoA-I appeared as a single band after electrophoresis on a homogeneous 20% SDS-polyacrylamide PhastGel (GE Healthcare) and staining with Coomassie Blue.

Production of recombinant apoE2, apoE3, and apoE4

ApoE2, apoE3, and apoE4 were expressed from *Escherichia coli* and purified by gel-permeation chromatography on a column of Sephacryl S-300 (GE Healthcare) (30, 38). The isolated apoE appeared as a single band when electrophoresed on a homogeneous 20% SDS-polyacrylamide PhastGel and stained with Coomassie Blue.

Isolation of LCAT

LCAT was isolated from samples of pooled, human plasma as described (39) and concentrated 10-fold using an Amicon® 8200 stirred ultrafiltration cell (Millipore Corp., Bedford, MA). LCAT activity was assessed using discoidal rHDL containing POPC, unesterified cholesterol (UC), and apoA-I radiolabeled with a tracer amount of [1α,2α-^{3}H]cholesterol ([^{3}H]UC) (GE Healthcare) as the substrate (40). The assay was linear as long as <30% of the [^{3}H]UC was esterified. The LCAT used in this study generated 4,414 nmol of cholesteryl ester (CE)/ml LCAT/h.

Isolation of PLTP

PLTP was isolated from samples of pooled, human plasma as described (41). Its activity was determined as the transfer of (1-^{14}C]1,2-dipalmitoyl L-3-phosphatidylcholine ([^{14}C]DPPC) (GE Healthcare) from small unilamellar POPC vesicles to HDL (42). The activities of the PLTP preparations used in this study ranged from 2.6 to 11.0 μmol phospholipid transferred/ml PLTP/h. All of the preparations were free of cholesteryl ester transfer protein (CETP) and LCAT activities and appeared as a single band after electrophoresis on a homogeneous 20% SDS-polyacrylamide PhastGel (GE Healthcare) and silver staining.

Preparation of spherical (A-I)rHDL, (E2)rHDL, (E3)rHDL, and (E4)rHDL

Discoidal rHDL containing POPC, UC, and apoA-I, apoE2, apoE3, or apoE4 were prepared by the cholate dialysis method (43) and converted into spherical rHDL by incubation at 37°C for 24 h with LDL and LCAT (30, 31). The rHDL were isolated by ultracentrifugation at 4°C in the $1.07 < d < 1.21$ g/ml density range and dialyzed extensively against TBS (10 mM Tris-HCl and 150 mM NaCl, pH 7.4) containing 1 mM EDTA-Na_2 and 0.01% (w/v) NaN_3 before use.

Time course of the remodeling of spherical (A-I)rHDL, (E2)rHDL, (E3)rHDL, and (E4)rHDL by PLTP

Spherical (A-I)rHDL, (E2)rHDL, (E3)rHDL, and (E4)rHDL were maintained at 4°C, incubated at 37°C for 24 h in the absence of PLTP, or incubated at 37°C for 1, 3, 6, 12, and 24 h in the presence of PLTP. When the incubations were complete, the rHDL were isolated by ultracentrifugation at a density of 1.25 g/ml with a single 16 h, 100,000 rpm spin in a TLA 100.1 rotor (Beckman Instruments) maintained at 4°C. The particle size distributions of the rHDL were determined by nondenaturing gradient gel electrophoresis.

Characterization of the conversion products generated by the PLTP-mediated remodeling of spherical (A-I)rHDL, (E2)rHDL, (E3)rHDL, and (E4)rHDL

Spherical (A-I)rHDL, (E2)rHDL, (E3)rHDL, and (E4)rHDL were either maintained at 4°C or incubated at 37°C for 24 h in the absence or presence of PLTP. When the incubations were complete, the rHDL were isolated by ultracentrifugation in the $1.063 < d < 1.25$ g/ml density range with a single 16 h spin at each of the upper and lower densities. The ultracentrifugally isolated rHDL were concentrated with an Amicon® Ultra-15 centrifugal filter device (Millipore) and applied to a Superose-6 column (GE Healthcare) attached to an ÄKTA FPLC system. The rHDL were eluted from the column at a flow rate of 0.5 ml/min. Fractions were collected at 1 min intervals.

Phospholipid transfers

[^{14}C]DPPC-labeled small unilamellar POPC vesicles were mixed with spherical (A-I)rHDL, (E2)rHDL, (E3)rHDL, or (E4)rHDL and incubated at 37°C for 1, 3, 5, 10, and 20 min in the presence or absence of PLTP. The vesicles were precipitated with a $MnCl_2$/heparin solution (42), and the [^{14}C]DPPC content of the rHDL in the supernatant was determined by liquid scintillation counting (Tri-Carb 21000 TR Liquid Scintillation Analyzer;

Packard Instrument Co., Downers Grove, IL). Precipitation of the vesicles with $MnCl_2$/heparin was essentially quantitative, with >95% of the rHDL remaining in solution.

Other methods

Nondenaturing 3–40% polyacrylamide gradient gel electrophoresis was used to quantitate rHDL size. The gels were stained with Coomassie Blue and scanned with a Sharp JX-610 scanner. Imagemaster software was used to quantitate particle size by reference to high molecular weight standards of known diameter (GE Healthcare).

The electrophoretic mobilities of the lipid-free apolipoproteins and spherical rHDL were determined by agarose gel electrophoresis using the following equation (44):

$$\text{mobility} = \frac{\text{migration distance } (\mu\text{m})/\text{time (s)}}{\text{voltage (V)}/\text{length of gel (cm)}} \qquad (Eq.\,1)$$

Mobilities were corrected for isoelectric point-dependent retardation as described (44).

All compositional analyses were carried out on a Roche Diagnostics/Hitachi 902 automatic analyzer (Roche Diagnostics GmbH, Mannheim, Germany). The bicinchoninic acid method was used to measure protein concentrations (45). Phospholipid concentrations were determined enzymatically (46). A Roche Diagnostics kit was used for the total cholesterol assays. UC concentrations were determined enzymatically (47). CE concentrations were calculated as the difference between the total and UC concentrations.

Statistical analysis

Two-way ANOVA (GraphPad Prism 4.0; GraphPad Software, Inc., San Diego, CA) was used to determine whether differences between phospholipid transfers were significant. Significance was set at $P < 0.05$.

RESULTS

Physical characterization of spherical rHDL

Although the spherical apoE-containing rHDL and the spherical (A-I)rHDL both contained three apolipoprotein molecules per particle (see Table 3 below) and were similar in terms of core lipid content, the apoE-containing rHDL (diameter, 10.2 nm) were significantly larger than the (A-I)rHDL (diameter, 9.1 nm) (**Fig. 1**). This is in agreement with what we have reported previously (30, 31, 48). Although this observation can be explained in terms of apoE being larger than apoA-I and therefore not accommodated as readily on the surface of a smaller HDL particle, it may also reflect the interaction of apoE with the charged phospholipid head groups on the rHDL surface in a way that is independent of particle core lipid content (49). As reported previously, the phospholipid-apolipoprotein molar ratio of all of the apoE-containing rHDL was greater than that of the (A-I)rHDL (30, 31, 48).

Influence of apoE on the PLTP-mediated phospholipid transfers from small unilamellar vesicles to spherical rHDL

PLTP transferred phospholipids from [^{14}C]DPPC-labeled small unilamellar POPC vesicles to the spherical (A-I)rHDL (closed diamonds) more rapidly than to the spherical (E2)rHDL (open squares), spherical (E3)rHDL (open triangles), and spherical (E4)rHDL (closed circles) (**Fig. 2**) [$P < 0.0001$ for (A-I)rHDL vs. (E2)rHDL, (E3)rHDL, and (E4)rHDL]. The rate of phospholipid transfer was also isoform-specific, with transfers from the vesicles to (E2)rHDL being less than to either (E3)rHDL or (E4)rHDL [$P < 0.0001$ for (E2)rHDL vs. (E3)rHDL and (E4)rHDL]. As PLTP does not mediate phospholipid transfers from rHDL to vesicles (28), it follows that these differences cannot be explained in terms of the rHDL phospholipids altering the vesicle structure.

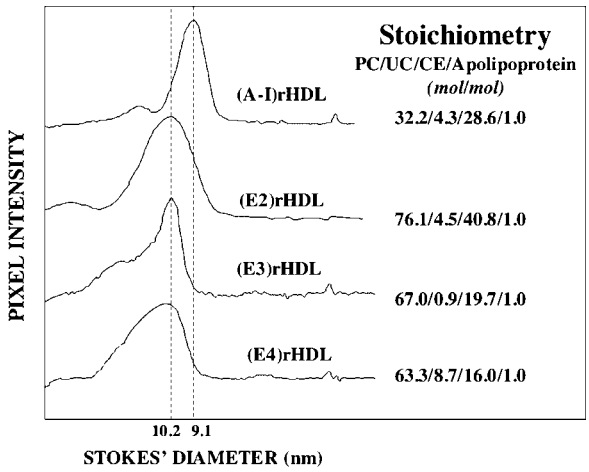

Fig. 1. Physical properties of spherical reconstituted high density lipoproteins (rHDL). Discoidal (E2)rHDL, (E3)rHDL, (E4)rHDL, and (A-I)rHDL were incubated at 37°C for 24 h with LDL and LCAT. The resulting spherical rHDL were isolated by ultracentrifugation, electrophoresed on a nondenaturing 3–40% polyacrylamide gradient gel, stained with Coomassie Blue, and scanned with a laser densitometer. Particle diameters were determined by reference to high molecular weight standards. Stoichiometries were calculated from triplicate determinations of the concentrations of individual constituents. CE, cholesteryl ester; PC, phosphatidylcholine; UC, unesterified cholesterol.

PLTP-mediated remodeling of spherical rHDL

Effects on particle size. ApoE- and apoA-I-containing spherical rHDL were incubated for 0–24 h in the presence or absence of PLTP. Changes in particle size were assessed by nondenaturing gradient gel electrophoresis (**Fig. 3**). Profiles A and B represent spherical rHDL that were either maintained for 24 h at 4°C or incubated at 37°C for 24 h in the absence of PLTP. Comparison of these profiles shows that incubation in the absence of PLTP does not affect rHDL size. The spherical (E2)rHDL, (E3)rHDL, and (E4)rHDL were progressively and quantitatively converted into large (diameter, 11.5–12.5 nm) and small (diameter, 7.8 nm) particles when they were incubated with PLTP for 1 h (profile C), 3 h (profile D), 6 h (profile E), 12 h (profile F), and 24 h (profile G). Approximately 75% of the spherical (A-I)rHDL were converted into large (diameter, 10.0 nm) and small (diameter, 7.7 nm) particles. This is consistent with what we reported previously (28).

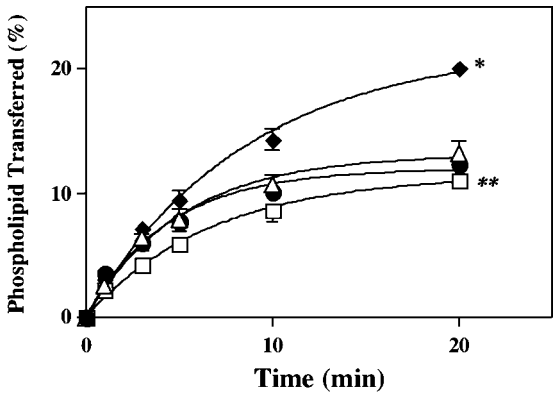

Fig. 2. Transfers of radiolabeled phospholipids from small unilamellar vesicles to spherical rHDL. [^{14}C]1,2-dipalmitoyl phosphatidylcholine (DPPC)-labeled small unilamellar POPC vesicles (final PL concentration, 1.5 μmol/ml) were mixed with spherical (A-I)rHDL (closed diamonds), (E2)rHDL (open squares), (E3)rHDL (open triangles), or (E4)rHDL (closed circles) (final apolipoprotein concentration, 0.625 mg/ml) and phospholipid transfer protein (PLTP) (final activity, 331 nmol phospholipid transferred/ml PLTP/h). The final volume of the incubation mixtures was 400 μl. The mixtures were incubated at 37°C for 1, 3, 5, 10, and 20 min. When the incubations were complete, the vesicles were precipitated with a $MnCl_2$/heparin solution and the [^{14}C]DPPC content of the rHDL was determined by liquid scintillation counting. Data points represent means ± SD of triplicate determinations. * $P < 0.0001$ for (A-I)rHDL versus (E2)rHDL, (E3)rHDL, and (E4)rHDL; ** $P < 0.0001$ for (E2)rHDL versus (E3)rHDL and (E4)rHDL.

The results of our previous study also established that the PLTP-mediated remodeling of spherical (A-I)rHDL into large and small particles caused lipid-free or lipid-poor apoA-I to dissociate from the particles (28). To determine whether this is also the case for apoE-containing rHDL, additional incubations were carried out for 0–24 h in the presence and absence of PLTP. When these incubation mixtures were subjected to nondenaturing gradient gel electrophoresis and immunoblotted for apoE, lipid-free/lipid-poor apoE was not detected in any of the samples (data not shown).

Characterization of the large and small conversion products. To determine whether PLTP remodels apoE-containing rHDL by a mechanism similar to what we reported previously for spherical (A-I)rHDL, the large and small conversion products were isolated and characterized. This was achieved by subjecting rHDL that had either been maintained at 4°C or incubated at 37°C for 24 h in the presence or absence of PLTP to gel-permeation chromatography. Phospholipid (closed squares), UC (closed triangles), CE (closed circles), and apolipoprotein (open diamonds) elution profiles are shown in **Fig. 4**. The rHDL that were either maintained at 4°C (Fig. 4A) or incubated at 37°C for 24 h in the absence of PLTP (Fig. 4B) eluted as a single peak. The rHDL that were incubated at 37°C for 24 h in the presence of PLTP eluted as two distinct peaks (Fig. 4C). Fractions were pooled as indicated and subjected to nondenaturing gradient gel electrophoresis (**Fig. 5**). Profiles A and B represent rHDL that were either maintained at 4°C or incubated at 37°C for 24 h in the absence of PLTP. Profiles C and D show the large and small conversion products, respectively.

The stoichiometries of the pooled samples are shown in **Table 1**. Incubation in the absence of PLTP had no effect on rHDL composition. The stoichiometries of the large and small (A-I)rHDL conversion products were comparable to what has been reported previously (28). The small apoE-containing conversion products were all deficient in UC, and their core lipid content was reduced markedly compared with the unmodified rHDL. The CE/apoE molar ratio of the large conversion products was increased relative to the original rHDL. As shown in Table 1, the recoveries of the individual rHDL constituents are essentially quantitative. This is consistent with PLTP not mediating the dissociation of apoE from the rHDL and confirms what was observed when the unprocessed incubation mixtures were subjected to nondenaturing gradient gel electrophoresis and immunoblotted for apoE (see above). The reduced recovery of apoA-I in the (A-I)rHDL that were incubated with PLTP reflects the dissociation of lipid-free/lipid-poor apoA-I from the particles (28).

Agarose gel electrophoresis was used to assess the surface charge of the original rHDL and the large and small conversion products (**Table 2**). The electrophoretic mobilities of lipid-free apoA-I, which migrates to a preβ position, plasma HDL, which has α migration, and plasma LDL, which migrates to a β position, are also presented. The lipid-free apoE, apoE3, and apoE4 all migrated to a β position, whereas the spherical (A-I)rHDL exhibited α migration. The spherical (E2)rHDL, (E3)rHDL, and (E4)rHDL that were either maintained at 4°C or incubated at 37°C in the absence of PLTP, as well as the large, apoE-containing conversion products, migrated to a position intermediate between that of α-migrating HDL and preβ-migrating lipid-free apoA-I. The surface charges of the spherical (E2)rHDL and (E4)rHDL were not uniform, with some of the particles migrating slightly faster (i.e., more negatively charged) than others. This suggests that apoE associates with these particles in more than one conformation. The small apoE-containing conversion products all migrated to an α position, whereas the small apoA-I-containing conversion products migrated to a preβ position.

Mechanism of the PLTP-mediated remodeling of spherical, apoE-containing rHDL

To elucidate the mechanism of the PLTP-mediated remodeling of apoE-containing spherical rHDL, it was first necessary to determine how many apoE molecules were associated with the large and small conversion products. In the case of the (A-I)rHDL, we reported previously that the large and small conversion products contain four and two molecules of apoA-I, respectively (28). We also reported that the spherical apoE-containing rHDL have three apoE molecules per particle and are remodeled by CETP into fusion products with six apoE molecules per particle (31). As incubation with PLTP increased the size of the apoE-containing rHDL to a similar extent to what was observed for the incubations with CETP (31), it is rea-

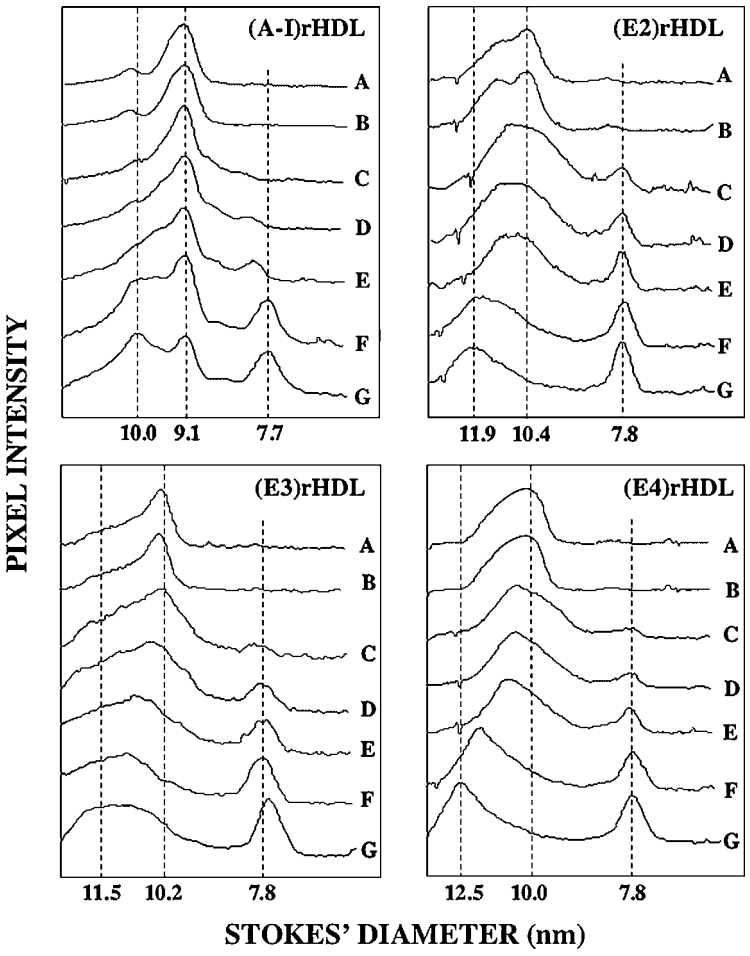

Fig. 3. Time course for the PLTP-mediated remodeling of spherical rHDL into large and small particles. Spherical (A-I)rHDL, (E2)rHDL, (E3)rHDL, and (E4)rHDL (final apolipoprotein concentration, 92.5 μg/ml) were either maintained at 4°C (profile A) or incubated at 37°C for 24 h in the absence (profile B) or for 1 h (profile C), 3 h (profile D), 6 h (profile E), 12 h (profile F), or 24 h (profile G) in the presence of PLTP (final activity, 7.1 μmol phospholipid transferred/ml PLTP/h). The final volume of the incubation mixtures was 0.2 ml. When the incubations were complete, the rHDL were isolated by ultracentrifugation and subjected to nondenaturing polyacrylamide gradient gel electrophoresis as described in the legend to Fig. 1.

sonable to assume that the large conversion products in the present study are also fusion products with six apoE molecules per particle. Furthermore, as the remodeling of the apoE-containing rHDL by PLTP did not mediate the dissociation of lipid-free or lipid-poor apoE from the particles, it also follows that the small conversion products must contain two molecules of apoE per particle.

The validity of these assumptions was assessed using the compositional data in Table 1 and the Stokes' diameters in Fig. 5 to calculate the particle volumes of the original rHDL and the large and small conversion products (see **Table 3** legend for details). As the results obtained by these approaches were in close agreement (Table 3), we elucidated a mechanism for the PLTP-mediated remodeling of apoE-containing rHDL using the spherical (E4)rHDL as an example (**Fig. 6**).

This mechanism is based on the assumption that, as reported previously for spherical (A-I)rHDL (28), PLTP initially interacts with two rHDL particles to generate a fusion product with six molecules of apoE [Fig. 6, step (i)]. The fusion product undergoes a structural rearrangement into a small conversion product with two molecules of apoE and an intermediate-sized particle with four apoE molecules [step (ii)]. The intermediate particle interacts with PLTP and another unmodified rHDL, generating a fusion product with seven apoE molecules [step (iii)]. As before, this fusion product undergoes a structural rearrangement into a small conversion product with two apoE molecules [step (iv)] and an intermediate-sized particle with five apoE molecules. The intermediate particle interacts with PLTP and another unmodified rHDL to generate a fusion product with eight apoE molecules [step (v)], which is remodeled into small and large conversion products that have two and six molecules of apoE, respectively [step (vi)]. This pathway is consistent with the results shown in Fig. 3, which indicate that the small conversion products accumulate in the incubation mixture before the large conversion products and that intermediate particles are generated during the remodeling. The CE/apoE molar ratios of the large and small conversion products in Fig. 6 are also consistent with the compositional data for (E4)rHDL in Table 1. It could be argued that PLTP may interact with two of the intermediate particles from step (ii) to generate a fusion product with eight apoE molecules and that this fusion product is converted directly into large and small particles [step (vi)]. However, the likelihood of this pathway making a significant contribution to the remodeling is low because it involves the simultaneous formation of large and small conversion products, which is not consistent with the results in Fig. 3.

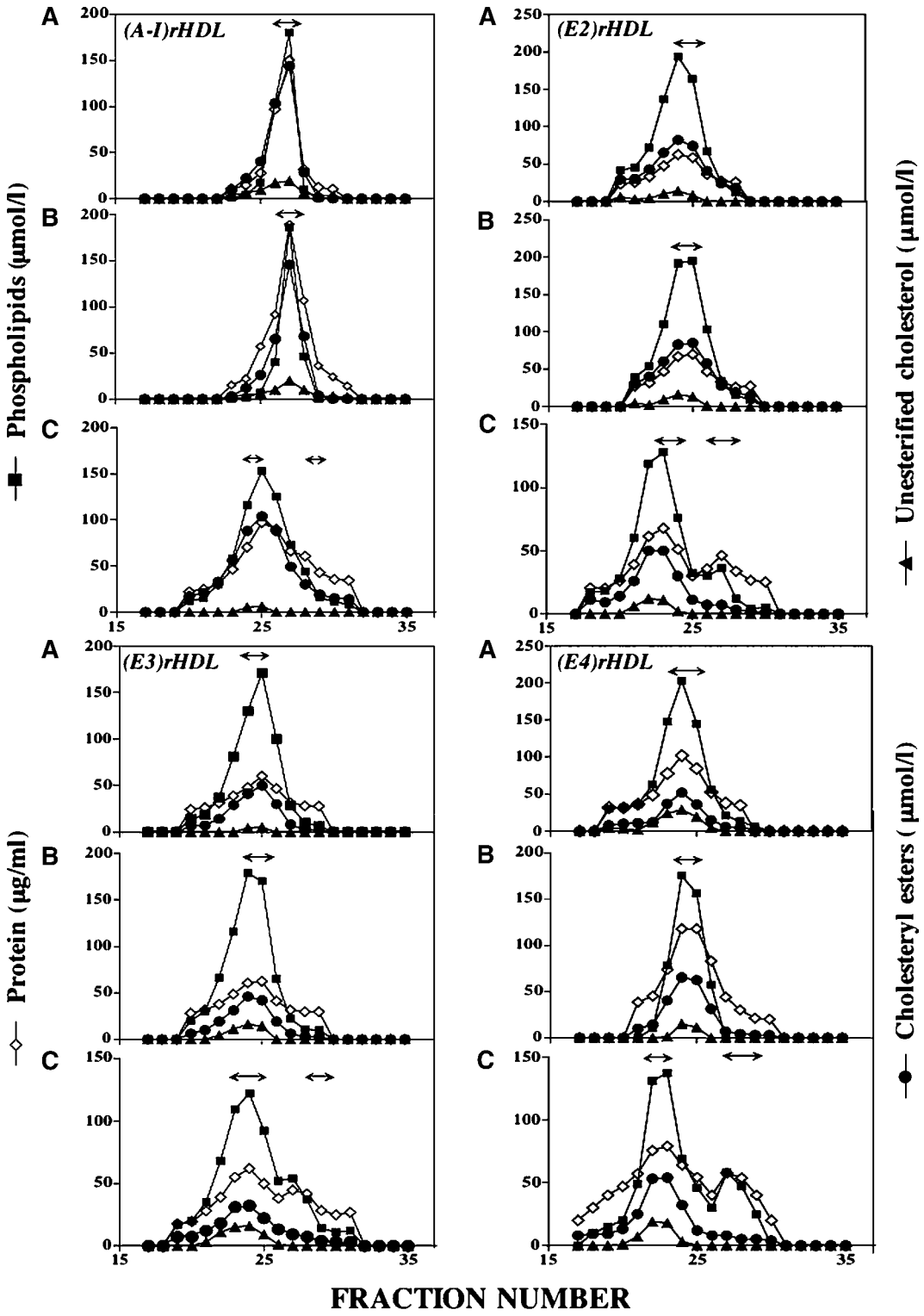

Fig. 4. Gel-permeation chromatographic elution profiles of spherical rHDL after incubation in the presence or absence of PLTP. Spherical (A-I)rHDL, (E2)rHDL, (E3)rHDL, and (E4)rHDL were either maintained at 4°C or incubated at 37°C for 24 h in the presence or absence of PLTP as described in the legend to Fig. 3. The final volume of the incubation mixtures was 7.1 ml. When the incubations were complete, the rHDL were isolated by ultracentrifugation, concentrated by ultrafiltration, and subjected to gel-permeation chromatography on a Superose-6 column as described in Experimental Procedures. Phospholipid (closed squares), unesterified cholesterol (closed triangles), cholesteryl ester (closed circles), and apolipoprotein (open diamonds) elution profiles are shown. Each data point represents the mean of triplicate determinations that varied by <6%. Selected fractions were pooled as indicated (↔).

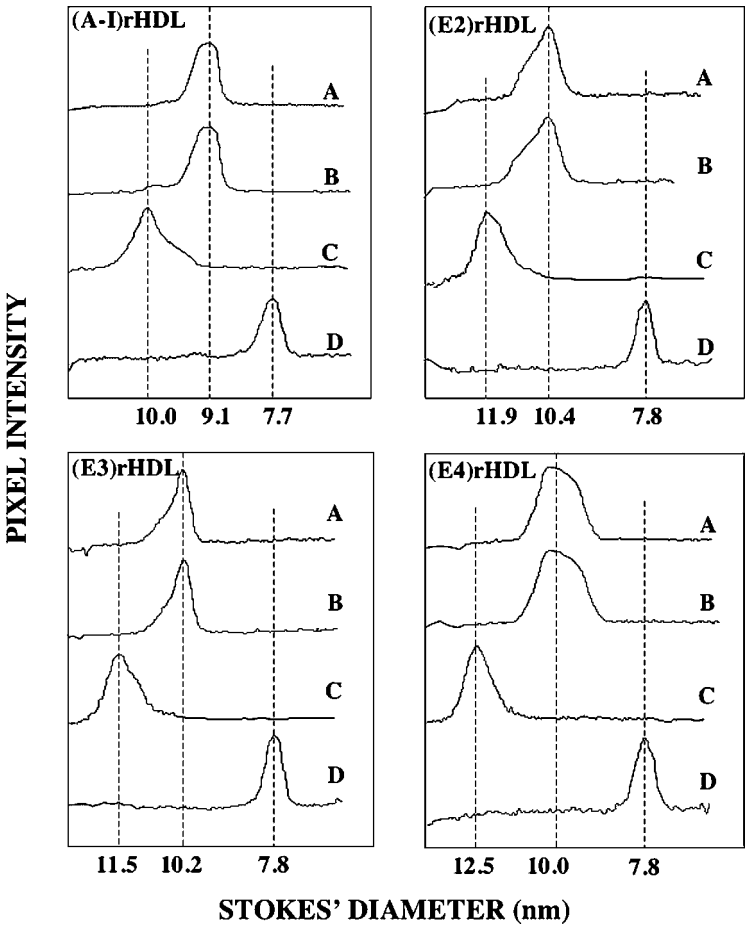

Fig. 5. Size distribution of the pooled gel-permeation chromatographic fractions. Spherical (A-I)rHDL, (E2)rHDL, (E3)rHDL, and (E4)rHDL were incubated in the presence or absence of PLTP and subjected to gel-permeation chromatography as described for Fig. 4. The pooled fractions from the gel chromatography column were subjected to nondenaturing polyacrylamide gradient gel electrophoresis. Profiles A and B show rHDL that were either maintained at 4°C or incubated at 37°C for 24 h in the absence of PLTP, respectively. Profiles C and D represent the large and small conversion products, respectively, that were generated when the rHDL were incubated for 24 h with PLTP.

DISCUSSION

The present study investigates the PLTP-mediated remodeling of apoE-containing HDL. The metabolism of apoE-containing HDL is becoming an issue of increasing importance, given that the concentration of these particles is likely to increase when HDL levels are increased by pharmacological inhibition of CETP. Under these circumstances, the remodeling of apoE-containing HDL will be dominated by PLTP. At present, nothing is known about the interaction of apoE-containing HDL with PLTP. This is largely because of the difficulties associated with isolating sufficient amounts of apoE-containing HDL from human plasma to carry out such studies. We have overcome this problem in the present study by using recombinant apoE to prepare spherical rHDL. The results show that PLTP remodels apoE-containing HDL into large and small particles via a series of particle fusions and structural rearrangements that do not involve the dissociation of apoE. These events are strikingly different from what has been reported for the PLTP-mediated remodeling of apoA-I-containing HDL, which involves a single particle fusion and the dissociation of lipid-free or lipid-poor apoA-I (27, 28).

The inability of apoE to dissociate from spherical rHDL may relate to the fact that it has a greater hydrophobic surface area than apoA-I (50) and that its C-terminal domain has a particularly high affinity for lipids (51). It is also possible that the dissociation of a molecule as large as apoE from HDL may severely compromise the structural integrity of the particles. The persistent association of apoE with rHDL is most likely responsible for the multiple structural rearrangements that occur during the remodeling of these particles by PLTP (Fig. 6). An additional factor that almost certainly contributes to these unusual structural rearrangements is that PLTP interacts with the large, but not the small, apoE-containing conversion products that are generated during the remodeling. Evidence that PLTP does not interact with the small conversion products comes from Fig. 2, which shows that the small conversion products accumulate in the incubation mixtures before the formation of the large conversion products. This is in contrast to what we have reported for the remodeling of spherical (A-I)rHDL, in which neither the large nor the small conversion products interact with PLTP (28). One reason why PLTP may interact with large apoE-containing, but not with large apoA-I-containing, conversion products is that the reduced surface curvature of the apoE-containing particles can accommodate the boomerang-shaped PLTP molecule more readily than the smaller, more highly curved apoA-I-containing rHDL. As both lipid binding pockets of PLTP are essential for full expression (52), it is reasonable to assume that they both must be in contact with the lipoprotein surface for remodeling to occur.

TABLE 1. Stoichiometry of the large and small conversion products generated by the PLTP-mediated remodeling of spherical rHDL

Spherical rHDL	Incubation Conditions	Stoichiometry PL/UC/CE/Protein	Recovery			
			PL	UC	CE	Protein
		mol/mol		%		
(A-I)rHDL	−PLTP, 4°C, 24 h	32.2/4.3/28.6/1.0	100	100	100	100
	−PLTP, 37°C, 24 h	27.8/3.0/21.8/1.0	88.6	89.6	93.4	100
	+PLTP, 37°C, 24 h		100	73	120	85.2
	Large particles	44.7/1.9/32.9/1.0				
	Small particles	20.6/0.0/14.0/1.0				
(E2)rHDL	−PLTP, 4°C, 24 h	76.1/4.5/40.8/1.0	100	100	100	100
	−PLTP, 37°C, 24 h	68.1/4.1/37.9//1.0	99.1	100	102.7	110.6
	+PLTP, 37°C, 24 h		93.5	68.9	102	114.8
	Large particles	57.7/3.4/45.2/1.0				
	Small particles	28.7/0.0/13.4/1.0				
(E3)rHDL	−PLTP, 4°C, 24 h	67.0/0.9/19.7/1.0	100	100	100	100
	−PLTP, 37°C, 24 h	58.8/3.8/18.9/1.0	115.8	100	97.4	110.7
	+PLTP, 37°C, 24 h		101.8	122	83.7	109.3
	Large particles	67.8/9.3/23.2/1.0				
	Small particles	35.8/0.0/7.6/1.0				
(E4)rHDL	−PLTP, 4°C, 24 h	63.3/8.7/16.0/1.0	100	100	100	100
	−PLTP, 37°C, 24 h	68.4/4.2/14.0/1.0	91.9	125.1	100	109.7
	+PLTP, 37°C, 24 h		85.3	100	102.5	114.9
	Large particles	59.2/8.2/23.7/1.0				
	Small particles	30.1/0.0/4.9/1.0				

CE, cholesteryl ester; PL, phospholipid; PLTP, phospholipid transfer protein; rHDL, reconstituted high density lipoprotein; UC, unesterified cholesterol. The rHDL were incubated at 37°C for 24 h in the presence or absence of PLTP and subjected to gel-permeation chromatography as described in the legend to Fig. 4. The composition of the pooled gel chromatographic fractions was determined as described in Experimental Procedures. Stoichiometries represent means of triplicate determinations that varied by <10%. Recoveries were calculated relative to the values obtained for control samples maintained for 24 h at 4°C in the absence of PLTP.

This, however, is not the case for the small conversion products. Irrespective of whether these particles contain apoE or apoA-I, their early accumulation in the incubations indicates that, once formed, they do not interact further with PLTP. This may be because PLTP cannot associate with the highly curved lipid-water interfacial regions of these small particles. It is also possible, however, that PLTP does bind to these small particles but that

TABLE 2. Surface charges of the large and small conversion products generated during the PLTP-mediated remodeling of spherical rHDL

Spherical rHDL	Incubation Conditions	Electrophoretic Mobility	Migration Characteristics
		$(\mu M \cdot s^{-1})/(V \cdot cm^{-1})$	
(A-I)rHDL	−PLTP, 4°C, 24 h	−0.504	α
	−PLTP, 37°C, 24 h	−0.504	α
	+PLTP, 37°C, 24 h		
	Large particles	−0.508	α
	Small particles	−0.523	Preβ
Lipid-free apoA-I (plasma HDL)		−0.364 (−0.512)	Preβ (α)
(E2)rHDL	−PLTP, 4°C, 24 h	−0.482, −0.416	Preβ/α
	−PLTP, 37°C, 24 h	−0.482, −0.416	Preβ/α
	+PLTP, 37°C, 24 h		
	Large particles	−0.460	Preβ/α
	Small particles	−0.504	α
Lipid-free apoE2 (plasma LDL)		−0.356 (−0.353)	β (β)
(E3)rHDL	−PLTP, 4°C, 24 h	−0.471	Preβ/α
	−PLTP, 37°C, 24 h	−0.471	Preβ/α
	+PLTP, 37°C, 24 h		
	Large particles	−0.497	Preβ/α
	Small particles	−0.504	α
Lipid-free apoE3		−0.349	β
(E4)rHDL	−PLTP, 4°C, 24 h	−0.497, −0.430	Preβ/α
	−PLTP, 37°C, 24 h	−0.482, −0.416	Preβ/α
	+PLTP, 37°C, 24 h		
	Large particles	−0.475	Preβ/α
	Small particles	−0.508	α
Lipid-free apoE4		−0.345	β

ApoA-I, apolipoprotein A-I. Spherical rHDL were incubated in the presence or absence of PLTP and processed as described in the legends to Figs. 3 and 4, then subjected to agarose gel electrophoresis. Electrophoretic mobilities were calculated according to equation 1.

TABLE 3. Volumes of the large and small conversion products generated by the PLTP-mediated remodeling of spherical rHDL

rHDL	Incubation Conditions	Stokes' Diameter	Apolipoproteins per Particle	Particle Volume (Observed)	Particle Volume[a] (Calculated)
		nm		*nm*^{3}	
(A-I)rHDL	−PLTP, 4°C, 24 h	9.1	3[b]	394.6	328.6
	−PLTP, 37°C, 24 h	9.1	3[b]	394.6	285.7
	+PLTP, 37°C, 24 h				
	Large particles	10.0	4[c]	523.6	515.3
	Small particles	7.7	2[c]	239.0	150.4
(E2)rHDL	−PLTP, 4°C, 24 h	10.4	3[b]	589.0	555.5
	−PLTP, 37°C, 24 h	10.2	3[b]	555.6	514.6
	+PLTP, 37°C, 24 h				
	Large particles	11.9	6	882.3	997.0
	Small particles	7.8	2	248.5	182.9
(E3)rHDL	−PLTP, 4°C, 24 h	10.2	3[b]	555.6	442.9
	−PLTP, 37°C, 24 h	10.2	3[b]	555.6	414.4
	+PLTP, 37°C, 24 h				
	Large particles	11.5	6	796.3	946.7
	Small particles	7.8	2	248.5	187.8
(E4)rHDL	−PLTP, 4°C, 24 h	10.0	3[b]	523.6	430.8
	−PLTP, 37°C, 24 h	10.3	3[b]	572.1	434.9
	+PLTP, 37°C, 24 h				
	Large particles	12.5	6	1,022.6	880.7
	Small particles	7.8	2	248.5	167.6

Observed particle volumes for the rHDL were determined from the Stokes' diameters in Fig. 5. The stoichiometries in Table 1 were used to obtain the calculated particle volumes. The calculations were based on the unmodified rHDL containing three apolipoprotein molecules per particle (28, 31) and assuming that the large and small apoE-containing conversion products contained six and two apoE molecules per particle, respectively. The large and small apoA-I-containing conversion products contain four and two apoA-I molecules per particle, respectively (28).

[a] Calculated from stoichiometries (Table 1). Volumes of the individual constituents were calculated assuming partial specific volumes for POPC, UC, CEs, and apolipoproteins of 0.97, 0.968, 1.044, and 0.705 ml/g, respectively (62). Total particle volumes were determined as the sum of the volumes of the individual constituents.

[b] Determined by cross-linking.

[c] From Ref. 28.

the phospholipids in the surface monolayer do not have ready access to the PLTP lipid binding domains. Irrespective of why this is the case, these observations are in keeping with those of Rao et al. (19), who reported that PLTP preferentially interacts with large, rather than small, HDL.

It is also noteworthy that the small conversion products that were generated during the PLTP-mediated remodeling of rHDL were markedly depleted of UC. Studies of plasma HDL have indicated that ~60% of the UC resides in the particle surface, whereas the remainder partitions into the core, most likely as a consequence of its displacement from the surface by the apolipoproteins (53). This suggests that areas on the lipoprotein surface that are in close proximity to the apolipoproteins are deficient in cholesterol. Furthermore, it gives an insight into the origins of the small conversion products by suggesting that they represent the dissociation from the fusion products of two apolipoprotein molecules, together with nearby phospholipids and some core lipid molecules.

The composition of the small, apoE-containing conversion products that were generated in the present study was unusual in that they contained only a few core lipid molecules. Although it could be argued that such a particle is likely to be discoidal rather than spherical, the recently reported structure of apoE/DPPC complexes, which are quasi-spheroidal, suggests that they are probably not discs (54, 55). Indeed, the notion that the small conversion products in the present study are spherical particles that consist of two juxtaposed horseshoe-shaped molecules of apoE that interact with the phospholipid head groups and shield a small number of CE molecules from the aqueous milieu in a micelle-type particle is appealing. The absence of such particles in vivo raises the possibility that they increase rapidly in size as they acquire phospholipids and cholesterol from cell membranes. Particles of this type are also likely to be converted into large, CE-rich spherical HDL by the action of LCAT.

It is possible that the large conversion products that are generated during the PLTP-mediated remodeling of HDL may also accept cholesterol and phospholipids from peripheral cells in a process that involves the ABC cassette transporter ABCG1 (56, 57). This appears to be the case for the large, apoE-containing HDL in the plasma of CETP-deficient subjects (58). When the possibility of the large and the small conversion products both acting as acceptors of cellular cholesterol and phospholipids is considered, it follows that the PLTP-mediated remodeling of apoE-containing HDL may play a significant role in cholesterol transport in the plasma and possibly also in the brain.

The present results also establish that PLTP transfers phospholipids to apoA-I-containing rHDL more readily

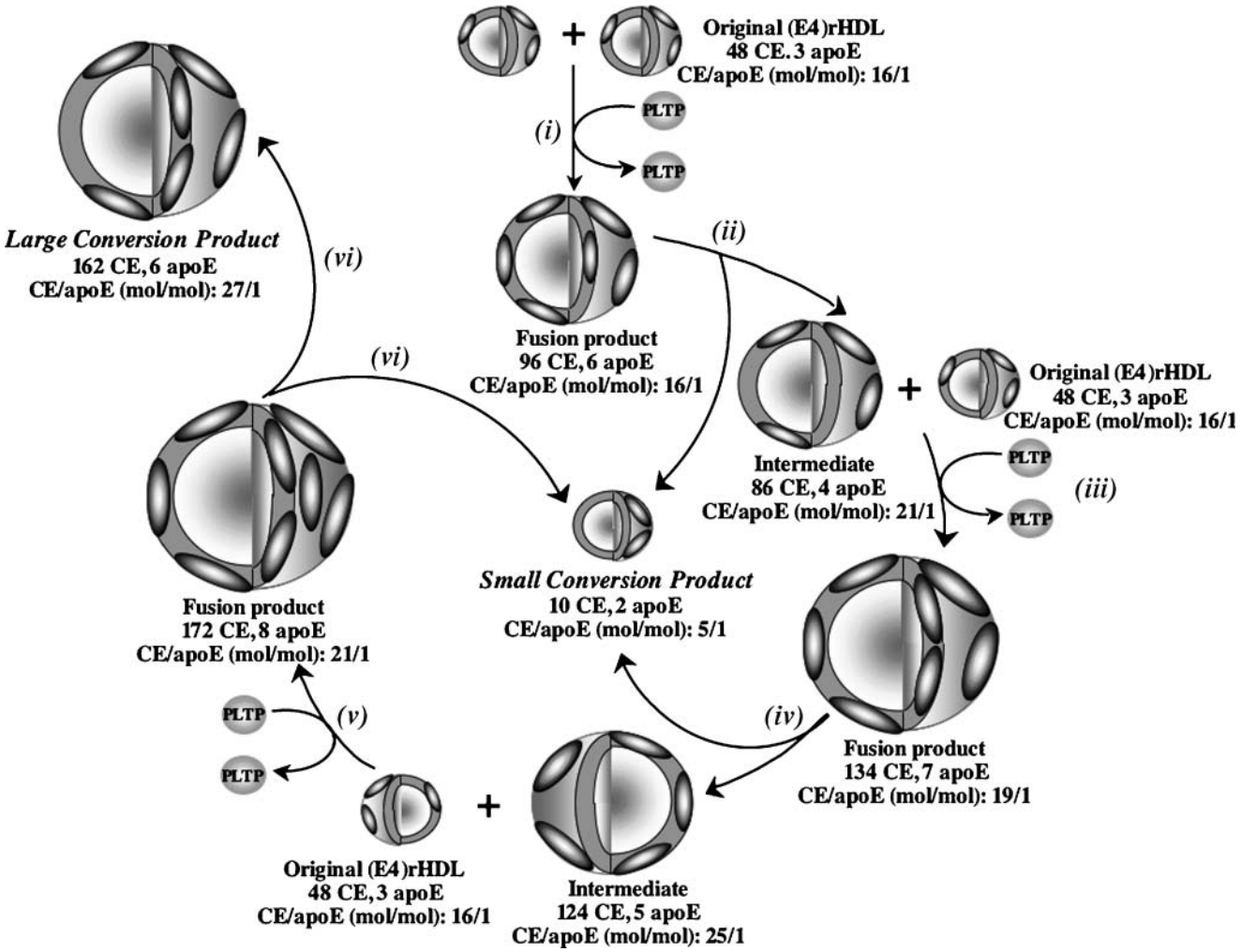

Fig. 6. Mechanism of the PLTP-mediated remodeling of spherical apolipoprotein E (apoE)-containing rHDL. Step (i): PLTP interacts with two rHDL particles to generate a fusion product with six molecules of apoE. Step (ii): A small particle with two molecules of apoE dissociates from the fusion product to generate an intermediate particle with four apoE molecules. Step (iii): The intermediate particle interacts with PLTP and an original rHDL particle, generating a fusion product with seven apoE molecules. Step (iv): The fusion product rearranges into a small particle with two molecules of apoE and an intermediate particle with five apoE molecules. Step (v): The intermediate rHDL interacts with PLTP and an original rHDL particle to generate a fusion product with eight apoE molecules. Step (vi): The fusion product with eight apoE molecules rearranges into large and small conversion products with two and six molecules of apoE, respectively.

than to apoE-containing rHDL. Although it is tempting to speculate that this may reflect the different surface charges of apoE- and apoA-I-containing rHDL, evidence showing that surface charge is not rate-limiting for phospholipid transfers indicates that this is unlikely to be the case (59). Given that the rate of phospholipid transfers increases with increasing surface curvature (60, 61), the enhanced PLTP-mediated phospholipid transfers to spherical (A-I)rHDL are more likely to reflect the smaller size of these particles relative to the apoE-containing rHDL.

In conclusion, the present results provide the first insight into the remodeling of apoE-containing HDL by PLTP. Given that the large as well as the small particles that are generated during the remodeling are potential acceptors of cellular phospholipids and cholesterol, it follows that this remodeling may be important for enhancing plasma cholesterol transport, especially under circumstances in which the activity of CETP is inhibited and the levels of apoE-containing HDL in the circulation are increased. If this is found to be the case, it follows that the interaction of PLTP with apoE-containing HDL may be of benefit in terms of reducing atherosclerosis. This category of HDL remodeling could also be important in the brain, where most of the cholesterol is transported in apoE-containing HDL and there is a high level of PLTP but little, if any, CETP activity. It is also possible that remodeling of apoE-containing HDL may offset the deleterious effects of the increased PLTP activity that has been reported in the plasma of subjects with type 2 diabetes (26).

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