Evidence That Cholesteryl Ester Transfer Protein-mediated Reductions in Reconstituted High Density Lipoprotein Size Involve Particle Fusion*

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It is well established that cholesteryl ester transfer protein (CETP) changes the size of high density lipoproteins (HDL) during incubation in vitro. It has been suggested that HDL-CETP-HDL ternary complex formation is involved in these changes. The present results, which are consistent with CETP changing the size of spherical reconstituted HDL (rHDL) by a mechanism involving fusion, support the ternary complex hypothesis. When rHDL containing a core of cholesteryl esters and either three molecules of apolipoprotein (apo) A-I/particle, (A-I)rHDL, or six molecules of apoA-II/particle, (A-II)rHDL, were incubated individually with CETP, their respective diameters decreased from 9.4 to 7.8 nm and from 9.8 to 8.8 nm. The small (A-I)rHDL and (A-II)rHDL contained, respectively, two molecules of apoA-I/particle and four molecules of apoA-II/particle. As all of the rHDL lipids and apolipoproteins were quantitatively recovered at the end of the incubations, it was apparent that there was a 50% increase in the number of particles. This increase in the number of particles can be explained as follows: (i) sequential binding of two rHDL to CETP to generate a ternary complex, (ii) fusion of the rHDL in the ternary complex, and (iii) rearrangement of the fusion product into three small particles. Various spectroscopic techniques were used to show that the small rHDL were structurally distinct from the original rHDL. These results provide the first evidence that CETP mediates the fusion of spherical rHDL.

Several investigators have reported that cholesteryl ester transfer protein (CETP) changes the size of high density lipoproteins (HDL) during incubation in vitro (1, 2). At present, the mechanisms of these size changes are poorly understood. This issue is of considerable importance as there is evidence suggesting that large HDL are more protective against coronary heart disease (CHD) than small HDL (3).

Recent studies have implicated CETP as a potentially atherogenic protein. First, mice, which are normally resistant to CHD, develop atherosclerotic lesions when made transgenic for CETP (4). Second, humans who are CETP-deficient have high plasma concentrations of large HDL (5, 6), low concentrations of low density lipoproteins (LDL) (7), and a low incidence of CHD (8). Finally, CETP-deficient animals, such as the rat, are resistant to atherosclerosis (9). In spite of these findings, the mechanism of action of CETP is still poorly understood.

CETP transfers cholesteryl esters (CE) and triglycerides (TG) between HDL, LDL, and very low density lipoproteins (VLDL) (10). It also transfers core lipids between different HDL particles (11). It has been suggested that CETP promotes these transfers either by (i) acting as a shuttle (12) or (ii) forming lipoprotein-CETP-lipoprotein ternary complexes (13). According to the shuttle model, CETP binds to the surface of a donor lipoprotein and acquires CE and/or TG. The resulting CETP-neutral lipid complex dissociates from the donor lipoprotein, enters the aqueous phase, and then binds to another lipoprotein that accepts the CE and/or TG. Evidence supporting this mechanism has come from studies showing that CETP has binding sites for CE and TG and that the bound lipids can be transferred from CETP to LDL (14). Although the ternary complex hypothesis was proposed several years ago (13), such complexes have never been isolated. Thus, if ternary complexes are formed, they may be unstable and exist only transiently before rearranging rapidly into more stable particles. It is conceivable that particle fusion is involved in such rearrangements.

The present study provides strong evidence that CETP promotes particle fusion. When homogeneous populations of spherical reconstituted HDL (rHDL) containing either three molecules of apolipoprotein (apo) A-I/particle, (A-I)rHDL, or six molecules of apoA-II/particle, (A-II)rHDL, were incubated with CETP, smaller particles with two molecules of apoA-I or four molecules of apoA-II were formed. As neither lipids nor apolipoproteins dissociated from the rHDL during the incubation, it was apparent that there was a 50% increase in the number of particles. This increase in particle numbers could occur only if the rHDL sustained a major structural reorganization such as fusion. It is proposed that CETP mediates the fusion of two rHDL particles to form an unstable fusion product that subsequently reorganizes into three smaller particles.

**EXPERIMENTAL PROCEDURES**

*Preparation of Spherical (A-I)rHDL and (A-II)rHDL—Discoidal rHDL containing 1-palmitoyl-2-oleoylphosphatidylcholine (POPC), unesterified cholesterol (UC), and apoA-I were prepared by the cholate
dialysis method (15). Spherical (A-I)rHDL were prepared by incubating discoidal (A-I)rHDL with LDL and lecithin:cholesterol acyltransferase (LCAT) exactly as described in a recent publication from this laboratory (16). Spherical (A-II)rHDL were prepared by displacing all the apoA-I from spherical (A-I)rHDL with lipid-free apoA-II (16). All the rHDL were dialyzed extensively against 0.01M Tris-buffered saline (TBS; pH 7.4) containing 0.15M NaCl, 0.005%(w/v) EDTA-Na₂, and 0.006%(w/v) NaN₃ before use.

Purification of LCAT and CETP—LCAT was purified from pooled human plasma (Transfusion Service, Royal Adelaide Hospital) as described elsewhere (17). The preparation used in this study generated 227 nmol CE/ml/h. CETP was also prepared from pooled human plasma as described elsewhere (18) except that the first chromatographic step was carried out on a Macro-Prep hydrophobic interaction gel (Bio-Rad) in an XK 50/30 column at a flow rate of 10 ml/min. The CETP preparation had 12.5 units of activity/ml, where 1 unit is the transfer activity of 1 ml of pooled human lipoprotein-deficient plasma (18).

Incubations—All incubations were carried out in stoppered plastic tubes in a shaking water bath that was maintained at 37 °C. Non-incubated control samples were stored at 4 °C. Details of individual incubations and subsequent processing of the samples are described in the legend to Fig. 1. When the incubations were complete, the rHDL were isolated by ultracentrifugation as the d < 1.25 g/ml fraction and then electrophoresed on 3/35% non-denaturing polyacrylamide gradient gels and stained with Coomassie Blue G-250. The figure represents laser densitometric scans of the gradient gels.

**FIG. 1.** Time course of the changes in (A-I)rHDL and (A-II)rHDL size during incubation with CETP. (A-I)rHDL (final apoA-I concentration of 0.14 mg/ml) and (A-II)rHDL (final apoA-II concentration of 0.17 mg/ml) were incubated individually with TBS alone or with TBS and CETP. The mixtures containing TBS only were either maintained at 4 °C or incubated for 24 h at 37 °C. Mixtures containing (A-I)rHDL or (A-II)rHDL and CETP (final concentration of 5.4 units/ml) were incubated at 37 °C for 1, 3, 6, 12, or 24 h. The final volume of the incubation mixtures was 1.8 ml. When the incubations were complete, an aliquot of each mixture was subjected to immunoblot analysis (see Fig. 3). The remaining rHDL were isolated by ultracentrifugation as the d < 1.25 g/ml fraction and then electrophoresed on 3/35% non-denaturing polyacrylamide gradient gels and stained with Coomassie Blue G-250. The figure represents laser densitometric scans of the gradient gels.
Fusion of Reconstituted HDL by CETP

Composition of (A-I)rHDL and (A-II)rHDL after incubation in the presence and absence of CETP

(A-I)rHDL and (A-II)rHDL were incubated for 0–24 h with either TBS or TBS and CETP and isolated by ultracentrifugation as described in the legend to Fig. 1. Concentrations of individual components were determined in triplicate as described under “Experimental Procedures.” In all cases the interassay variation was less than 10%. Recoveries of individual constituents are expressed relative to the recovery of the non-incubated controls.

**TABLE I**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Incubation conditions</th>
<th>Additions</th>
<th>Stoichiometry</th>
<th>Recovery</th>
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<td></td>
<td></td>
<td>PL UC CE A-I A-II</td>
<td>PL UC CE A-I A-II</td>
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<tr>
<td>(A-I)rHDL</td>
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<td>TBS</td>
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<td>100 100 100 100 0</td>
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<td>103 119 100 95 0</td>
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<tr>
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<td>52.6 6.8 26.6 0 2.0</td>
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<td></td>
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<td>CE</td>
<td>55.6 7.2 28.0 0 2.0</td>
<td>101 104 100 104 0</td>
</tr>
</tbody>
</table>

a PL, phospholipid; UC, unesterified cholesterol; CE, cholesterol esters; A-I, apoA-I; A-II, apoA-II.

(19). Non-denaturing gradient gel electrophoresis was carried out on 335% or 340% polyacrylamide gradient gels (Gradipore, Sydney, Australia) as described (20).

**Spectroscopic Studies**—A Perkin-Elmer LS-50 luminescence spectrometer fitted with a thermostatted cell holder and polarizers was used for these studies. Emission spectra were recorded from 300 to 380 nm using an excitation wavelength of 295 nm for (A-I)rHDL and 280 nm for (A-II)rHDL. The same excitation wavelengths were used for the intrinsic polarization studies. All data were acquired at 25 °C, with the temperature being maintained by a Lauda RM6T recirculating water bath (Lauda-Königshofen, Germany). Details of the steady-state fluorescence polarization studies using aliquots of HDL labeled individually with 1,6-diphenyl-1,3,5-hexatriene (DPH), 1-(4-trimethylammoni- umphenyl)-6-phenyl-1,3,5-hexatriene p-toluene sulfonate (TMA-DPH), and 6-propionyl-2-dimethylaminonaphthalene (PRODAN) are described elsewhere (18).

The circular dichroism (CD) studies were carried out by Professor W. Sawyer, Department of Biochemistry, University of Melbourne, Australia. Details of sample preparation and data acquisition are described in an earlier publication (19).

**Other Techniques**—All chemical analyses were carried out on a Co-bas Faraf ultracentrifugal analyzer (Roche Diagnostics, Zurich, Switzerland) (17). Cross-linking was performed as described by Staros (21). For immunoblot analysis, aliquots of incubation mixtures, which had not been ultracentrifuged, were electrophoresed on 3/40% non-denaturing polyacrylamide gradient gels, transferred to nitrocellulose membranes, and incubated with sheep anti-human apoA-I or apoA-II antiserum (Boehringer Mannheim, Germany) (2). The membranes were developed by enhanced chemiluminescence (Amersham Life Science, Inc.).

**Statistical Analysis**—The Student’s t test for paired samples was used to determine whether differences between values were significant.

**RESULTS**

**Time Course of the Changes in (A-I)rHDL and (A-II)rHDL Size and Composition during the Incubation with CETP** (Figs. 1-3, Tables I and II)—The changes in rHDL size, which occurred during incubation with CETP, were assessed by non-denaturing gradient gel electrophoresis (Fig. 1). The respective diameters of the non-incubated (A-I)rHDL and (A-II)rHDL were 9.4 and 9.8 nm. The slightly larger size of the (A-II)rHDL relative to (A-I)rHDL is consistent with an earlier report from this laboratory (16). (A-I)rHDL and (A-II)rHDL size was not affected by 24 h of incubation with TBS or by incubation for 1 h with CETP. After 3 h of incubation with CETP, populations of larger and smaller (A-I)rHDL were apparent. At this time, a population of smaller particles was also evident as a shoulder on the descending limb of the (A-II)rHDL peak. At 6 h, the small (A-I)rHDL and (A-II)rHDL were more prominent. By 12 h, most of the (A-I)rHDL and (A-II)rHDL had been converted into smaller particles. After 24 h of incubation with CETP, conversion of the (A-I)rHDL and (A-II)rHDL into particles 7.8 and 8.8 nm in diameter, respectively, was quantitative.

The stoichiometry of the (A-I)rHDL and (A-II)rHDL after 0–24 h of incubation with CETP is shown in Table I. As two molecules of apoA-II are required to displace one molecule of apoA-I from (A-I)rHDL (22), the results for the (A-II)rHDL are expressed relative to two molecules of apoA-II. The lipid/protein molar ratios of the non-incubated samples were comparable. This shows that displacement of apoA-I by apoA-II does not modify HDL composition and is in agreement with what was reported earlier (16). The PL/UC/apoA-II molar ratio of the non-incubated (A-I)rHDL was 51.6/6.0/26.2/1.0. After 24 h of incubation with CETP, these ratios were essentially unchanged at 55.6/7.5/27.5/1.0. Comparable results were obtained for the (A-II)rHDL, with the PL/UC/apoA-II molar ratios of the (A-II)rHDL before and after 24 h of incubation with CETP being 57.4/7.2/29.2/2.0 and 55.6/7.2/28.2/0.0, respectively.

Given that the (A-I)rHDL and (A-II)rHDL size decreased during incubation with CETP, it is reasonable to assume that the number of lipid and protein molecules/particle also decreased. To determine whether the number of apolipoprotein molecules/particle had decreased, the (A-I)rHDL and (A-II)rHDL were incubated for 24 h with TBS or CETP, cross-linked, and subjected to SDS gradient gel electrophoresis (Fig. 2). Cross-linked, lipid-free apoA-I was used as a standard (Fig. 2, profile E). The (A-I)rHDL that were incubated with TBS and CETP contained, respectively, three (profile A) and two (profile B) molecules of apoA-I/particle. Following incubation with TBS, the (A-II)rHDL contained six molecules of apoA-II/particle (profile C). After 24 h of incubation with CETP, they contained four molecules of apoA-II/particle (profile D).

Western blotting was used to show that the reduction in the number of apolipoprotein molecules/particle was not caused by dissociation of apoA-I or apoA-II from the rHDL. Aliquots of unprocessed incubation mixtures were subjected to non-denaturing gradient gel electrophoresis followed by immunoblot analysis for apoA-I and apoA-II (Fig. 3). The apoA-I in the non-incubated (A-I)rHDL was confined to a single population of particles (lane 1), the size of which was not affected by 24 h of incubation with TBS (lane 2). After incubation with CETP, apoA-I was evident, initially, in larger and smaller particles (lanes 3–5). At 12 (lane 6) and 24 h (lane 7) of incubation, apoA-I was apparent only in small particles. A small amount of
lipid-free apoA-I was also detected at 24 h (lane 7). Lane 8 represents lipid-free apoA-I. In the non-incubated (A-II)rHDL sample, apoA-II was evident in a single population of particles (lane 9) whose size was not affected by incubation with TBS (lane 10). As with the (A-I)rHDL, apoA-II was present in larger and smaller particles after 1, 3, and 6 h of incubation with CETP (lanes 11–13). ApoA-II was present only in small particles at 12 h (lane 14) and 24 h (lane 15). A trace amount of lipid-free apoA-II was present in the incubation mixture at 24 h. Lane 16 represents lipid-free apoA-II.

The recovery data in Table I show that all of the apolipoproteins that were present in the original (A-I)rHDL and (A-II)rHDL were recovered quantitatively in the small particles that were formed following 24 h of incubation with CETP. This result confirms that the conversion to smaller particles was not caused by dissociation of lipid-free apoA-I or apoA-II from the original rHDL. Furthermore, as the rHDL lipid/protein molar ratios did not change during the incubation with CETP (Table I), it follows that the small particles not only contained fewer apolipoprotein molecules than the original rHDL, but they also contained fewer lipid molecules. The decrease in the number of lipid and protein molecules is shown in Table II.

Influence of CETP on the Physical Properties of (A-I)rHDL and (A-II)rHDL (Table III, Fig. 4)—The wavelength of maximum fluorescence of (A-I)rHDL was not affected by incubation with CETP (Table III). As this parameter reflects the environment of the Trp residues in the N-terminal region of apoA-I, it follows that the conformation of this part of the protein is independent of rHDL size. This conclusion is supported by the CD data, which show that incubation with CETP had no effect on the percent α-helix, β-turn, or random coil. In the case of (A-II)rHDL, incubation with CETP did not alter the wavelength of maximum fluorescence of the apoA-II Tyr residues. There was, however, a minor change in secondary structure, with the apoA-II in the smaller particles having a lower α-helical content and more random coil than the control (A-II)rHDL. The discrepancy between the (A-II)rHDL fluorescence and CD results is probably due to the fluorescence of the apoA-II Tyr residues being insensitive to conformational change.

The intrinsic steady-state polarization values reflect the local rotational motions of the apoA-I Trp residues in (A-I)rHDL and the apoA-II Tyr residues in (A-II)rHDL. When (A-I)rHDL and (A-II)rHDL were incubated for 24 h with CETP, their intrinsic polarization decreased. In other words, the local rotational motions of the apoA-I Trp and apoA-II Tyr residues increased when rHDL size decreased. The polarization of the (A-I)rHDL, which had been incubated with either TBS or CETP, was lower than that of lipid-free apoA-I. This shows that lipid association enhances the rotation of apoA-I Trp residues and is in agreement with what has been reported previously (17). On the other hand, after 24 h of incubation with either CETP or TBS, the polarization of lipid-associated apoA-II was decreased.
higher than that of lipid-free apoA-II. This is consistent with lipid association restricting the rotation of apoA-II Tyr residues.

The (A-I)rHDL and (A-II)rHDL, which had been incubated with TBS or CETP, were also subjected to agarose gel electrophoresis. This study was carried out in order to determine whether rHDL surface charge is influenced by particle size and the number of apolipoprotein molecules/particle. Sample migration is expressed in terms of electrophoretic mobility (Table III). The results show that incubation with CETP did not affect rHDL migration. In other words, the surface charge of rHDL is influenced neither by particle size nor by the number of apolipoprotein molecules/particle. It should also be noted that both the (A-I)rHDL and (A-II)rHDL migrated more rapidly than lipid-free apoA-I and apoA-II. This is consistent with lipid association increasing the negative charge of the apolipoproteins and confirms what has been reported previously (16).

Phospholipid acyl chain and head group packing order was assessed from the steady-state fluorescence polarization of (A-I)rHDL and (A-II)rHDL, which had been labeled, respectively, with DPH and TMA-DPH (Fig. 4). Lipid-water interfacial polarity was monitored as the wavelength of maximum fluorescence of PRODAN-labeled rHDL (Fig. 4). The closed and open symbols represent samples incubated with TBS and CETP, respectively. Incubation with CETP decreased both the DPH and TMA-DPH polarization values. This is consistent with the phospholipid acyl chains and head groups being less ordered in small rHDL. Apolipoprotein composition had no effect on phospholipid acyl chain packing as judged by the comparable DPH polarization values for (A-I)rHDL and (A-II)rHDL. The higher polarization of TMA-DPH in (A-I)rHDL relative to (A-II)rHDL is consistent with apoA-II increasing the order of the (A-II)rHDL phospholipid head groups.

Incubation with CETP had no effect on the wavelength of maximum fluorescence of PRODAN-labeled (A-I)rHDL. In other words, the lipid-water interfacial polarity of (A-I)rHDL was not influenced by the decrease in particle size. The wavelength of maximum fluorescence of these samples increased progressively above 20°C, showing that the polarity, or hydration, of the (A-I)rHDL lipid-water interface increased at higher temperatures. The wavelength of maximum fluorescence of the (A-II)rHDL, which were incubated with TBS, increased only when the temperature exceeded 35°C. This suggests that the lipid-water interface of (A-II)rHDL is more resistant to hydration than that of (A-I)rHDL. Incubation with CETP further increased the resistance of the (A-II)rHDL to hydration, with the wavelength of maximum fluorescence increasing above 40°C.

**DISCUSSION**

This study shows that CETP reduces the size of spherical (A-I)rHDL and (A-II)rHDL during incubation in vitro. When spherical (A-I)rHDL, containing three molecules of apoA-I/particle, and (A-II)rHDL, containing six molecules of apoA-II/particle, were incubated with CETP, there was (i) a reduction in rHDL size, (ii) a one-third decrease in the number of lipid and apolipoprotein in the small rHDL and, thus, (iv) a 50% increase in the number of rHDL particles. These findings can be explained by CETP mediating the fusion of two rHDL particles to form a large, unstable fusion product that rearranged rapidly into a larger number of small particles. This process is shown schematically in Fig. 5 and discussed in detail below.

The idea that fusion is involved in the conversion of (A-I)rHDL and (A-II)rHDL to small particles has important mechanistic implications. Several years ago, it was suggested that
CETP transfers CE and TG between lipoproteins either by acting as a shuttle (12) or by forming lipoprotein ternary complexes (13). Although there is good evidence showing that CETP shuttles lipids between lipoproteins (14), the ternary complex hypothesis has yet to be validated. Studies of protein-mediated membrane fusion have shown that the following events must occur prior to fusion: (i) formation of “bridges”, or ternary complexes, between the fusogen and closely apposed membranes (23, 24) and (ii) insertion of hydrophobic, amphipathic regions of the fusogen into the membranes (25, 26). Although membranes are bilayers, only their outer leaflets are involved in these processes. As such, it is likely that these events may also occur during lipoprotein fusion. Given that CETP is an extremely hydrophobic protein (27), which can insert into lipid monolayers (28), it follows that it may well have fusogenic properties and mediate the formation of intermediates such as ternary complexes.

Most fusogenic proteins have distinct, amphipathic amino acid sequences with hydrophobicity indexes ranging from 0.5 to 0.7 (29). Candidate fusogenic regions of CETP include the putative α-helix spanning amino acids 454–457 (30). These amino acids are in the highly conserved C-terminal part of CETP that contains binding sites for CE and TG (31). Amino acids 470–475, which bind CE and TG, but not PL, also constitute a potentially fusogenic region of CETP (31).

One problem that is encountered frequently in studies of this type is that the concentration of the fusion product is likely to be very low. The immunoblotting results in Fig. 3 show that large particles are formed when (A-I)rHDL and (A-II)rHDL are incubated with CETP from 1 to 6 h. The size of these particles is consistent with a fusion intermediate. However, these large particles were not apparent on the Coomassie-stained gradient gels in Fig. 1. As the lower limit of detection for Coomassie-stained proteins is approximately 0.5 μg (32), compared with less than 1 pg for antigens detected by enhanced chemiluminescence, it is likely that the concentration of the large particles was insufficient to be detected on Coomassie-stained gradient gels. Thus, it was not technically feasible to isolate the large particles in amounts sufficient for their characterization.

The mechanism by which CETP mediates reductions in the size of (A-I)rHDL and (A-II)rHDL must be able to account for the observation that two rHDL particles containing either three molecules of apoA-I or six molecules of apoA-II are converted into three particles that contain two molecules of apoA-I or four molecules of apoA-II. This transformation could not occur without substantial reorganization of the rHDL constituents in a process that is difficult to explain other than by particle fusion. A pathway that accounts for the reduction in size and number of lipid and apolipoprotein molecules as well as the increased particle numbers is shown in Fig. 5 for (A-I)rHDL. According to this scheme, fusion is initiated by the binding of CETP to (A-I)rHDL to form an (A-I)rHDL-CETP complex, step a. Another (A-I)rHDL binds to the complex to generate an (A-I)rHDL-CETP-(A-I)rHDL ternary complex, step b. The (A-I)rHDL in the ternary complex fuse to form a large, unstable fusion product to which CETP is still bound, step c. The fusion product rearranges into three small particles, step d. During this rearrangement, free CETP is released from the fusion product and re-enters the pathway by binding to another unmodified (A-I)rHDL, step e. The small particles that are formed in step d are not modified further by CETP and accumulate as end products of the pathway.

The recycling of CETP between a free and HDL-associated form is an important feature of the pathway. Under circumstances where the concentration of HDL exceeds that of CETP (such as in plasma), irreversible binding of CETP to the HDL would result in only a proportion of the particles changing in size. The plasma concentration of HDL is approximately 16 μM,
compared with 23 nM for CETP (33). This gives an HDL/CETP molar ratio of approximately 700/1. In other words there are approximately 700 HDL particles for each molecule of CETP in plasma. If CETP bound irreversibly to the HDL in plasma, less than 0.2% of the particles would change size. However, as CETP mediates major changes to the size of HDL during incubation in vitro (1, 2), it must interact with most, if not all, of the HDL in plasma. This can occur only if the CETP recycles between the free and HDL-associated form.

An earlier study from this laboratory showed that the size of rHDL decreased following incubation with CETP and the phospholipid/triglyceride emulsion, Intralipid (Kabi Pharmacia AB, Sweden) (18). In that study, the reduction in rHDL size was attributed to CETP-mediated transfers of core lipids out of the rHDL into Intralipid and the dissociation of one-third of the apoA-I, in the lipid-free form, from the rHDL (18). The current results show that CETP can also decrease the size of rHDL by a quite different mechanism. As Intralipid was not included in the present study, core lipids were not transferred out of the rHDL. The results also show that apoproteins did not dissociate from the rHDL during the incubation. Although lipid-free apoA-I and apoA-II were detected at 24 h in the immunoblots in Fig. 3, the amounts were minimal and were unlikely to have contributed to the reduction in rHDL size. Additional evidence that the reduction in particle size was not caused by dissociation of apolipoproteins from the rHDL is that lipid-free apoA-I and apoA-II were not detected by immunoblotting after 12 h of incubation with CETP at a time when most of the HDL had decreased in size. Finally, the results in Table I show that the recovery of apoA-I and apoA-II was comparable for the control rHDL and the rHDL, which had been incubated for 24 h with CETP.

In the present study, the small particles that were formed when (A-I)rHDL were incubated with CETP had a diameter of 7.8 nm (Fig. 1). Each of these small particles contained 55 molecules of CE (Table II). As the molecular volume of CE is approximately 1,129 Å³ (34), it follows that the core volume of these small CE-containing particles was 62,000 Å³. In the earlier study, where (A-I)rHDL were incubated with CETP and Intralipid (18), the resulting small particles were 8.0 nm in diameter and contained one molecule of CE and eight molecules of TG/particle. As the molecular volume of TG is 1,606 Å³ (34), the core volume of these particles was approximately 14,000 Å³. In other words, the core volume of the small CE-containing particles in the present study was approximately four times greater than that of the TG-containing conversion products in the earlier study (18). However, compositional analysis revealed that the total volumes of the small CE- and TG-containing rHDL were comparable at 278,000 and 291,000 Å³ respectively. The issue, therefore, arises as to how spherical rHDL, which are comparable in size and total volume, can have different core volumes. One possibility is that the CE- and TG-containing rHDL are structurally distinct.

An insight into the structural differences between small TG- and CE-containing rHDL was obtained by comparing the spectroscopic data from both studies. The DPH polarization results show that the small TG-containing rHDL in the earlier study had less ordered phospholipid acyl chains than the small CE-containing particles in the present study. This is consistent with the observations of other investigators who have found that phospholipid acyl chain order is decreased by TG (35, 36) and increased by CE (36). The TMA-DPH polarization results from the two studies were, by contrast, comparable. This suggests that CE and TG have similar effects on phospholipid head group packing. Given that the carbonyl groups of the CE and TG molecules that partition into the surface monolayer of rHDL are probably located close to the phospholipid head groups (37), this result is to be expected.

However, Hamilton and Small (38) have reported that TG carbonyl groups are located further from the surface of a bilayer than phospholipid carbonyl groups. If this is the case, it is
conceivable that water molecules can access the lipid-water interface of a TG-containing particle more readily than they can access the lipid-water interface of a CE-containing particle. The PRODAN results support this proposal. The lipid-water interfacial hydration of the CE-containing (A-I)rHDL in the current study is independent of particle size (Fig. 4). The lipid-water interfacial polarity of the TG-containing conversion products in the earlier study (18), by contrast, was enhanced relative to their CE-containing precursors. In other words, the surface of an rHDL that contains TG is more hydrated than the surface of a similarly sized particle that contains CE.

The conformation of the apoA-I in (A-I)rHDL is also influenced by core lipid composition. The wavelength of maximum fluorescence of small CE-containing (A-I)rHDL is 335 nm (Table III) compared with 332 nm for small TG-containing (A-I)rHDL (18). As this parameter monitors the environment of apoA-I Trp residues, which are all located in the N-terminal region of the protein, it follows that these residues are in a more hydrophobic environment in TG-containing rHDL.

In summary, the present study shows that spherical (A-I)rHDL and (A-I) HDL decrease in size during incubation with CETP. It has been concluded that the reduction in size is caused by a structural rearrangement that may involve the formation of an unstable fusion intermediate. When these results are considered together with the results of our earlier study (18), it is apparent that CETP can regulate the size of HDL by at least two mechanisms during incubation in vitro. The results also suggest that the mechanism that operates at any given time is likely to be influenced by the presence of other lipoproteins.

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