Phospholipid transfer protein (PLTP) remodels high density lipoproteins (HDL) into large and small particles. It also mediates the dissociation of lipid-poor or lipid-free apolipoprotein A-I (apoA-I) from HDL. Remodeling is enhanced markedly in triglyceride (TG)-enriched HDL (Rye, K.-A., Jauhiainen, M., Barter, P. J., and Ehnholm, C. (1998) J. Lipid. Res. 39, 613–622). This study defines the mechanism of the remodeling of HDL by PLTP and determines why it is enhanced in TG-enriched HDL. Homogeneous populations of spherical re-constituted HDL (rHDL) containing apoA-I and either cholesteryl esters only (CE-rHDL; diameter 9.3 nm) or CE and TG in their core (TG-rHDL; diameter 9.5 nm) were used. After 24 h of incubation with PLTP, all of the TG-rHDL, but only a proportion of the CE-rHDL, were converted into large (11.3-nm diameter) and small (7.7-nm diameter) particles. Only small particles were formed during the first 6 h of incubation of CE-rHDL with PLTP. The large particles and dissociated apoA-I were apparent after 12 h. In the case of TG-rHDL, small particles appeared after 1 h of incubation, while dissociated apoA-I and large particles were apparent at 3 h. The composition of the large particles indicated that they were derived from a fusion product. Spectroscopic studies indicated that the apoA-I in TG-rHDL was less stable than the apoA-I in CE-rHDL. In conclusion, these results show that (i) PLTP mediates rHDL fusion, (ii) the fusion product rearranges by two independent processes into small and large particles, and (iii) the more rapid remodeling of TG-rHDL by PLTP may be due to the destabilization of apoA-I.

The Mechanism of the Remodeling of High Density Lipoproteins by Phospholipid Transfer Protein*

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Phospholipid transfer protein (PLTP) transfers phospholipids (PL) between high density lipoproteins (HDL) and very low density lipoproteins as well as between different particles within the HDL fraction (1, 2). It also remodels HDL into large and small particles in a process that is accompanied by the dissociation of lipid-poor or lipid-free apolipoprotein A-I (apoA-I) (3–8). Remodeling is enhanced markedly in HDL that contain triglyceride (TG) in their core (9).

Evidence of the importance of PLTP in HDL metabolism comes from studies of mice transgenic for human PLTP. These animals have increased levels of pre-β1-migrating HDL, the initial acceptors of cellular cholesterol in the first step of the reverse cholesterol pathway, and are also resistant to intracellular cholesterol accumulation (10–12). Studies of PLTP knock-out mice have shown that PLTP is essential for maintaining normal HDL levels in plasma (13). Moreover, it has been reported recently that PLTP-mediated transfers of phospholipids between HDL and other lipoprotein classes are not interchangeable with the phospholipid transfers that are mediated by cholesteryl ester transfer protein (CETP) (14).

The mechanism of the remodeling of HDL by PLTP is poorly understood. Although there is evidence that particle fusion and the dissociation of lipid-poor or lipid-free apoA-I are involved (7, 8), nothing is known about how the interaction of PLTP with HDL is regulated or the events that occur when HDL are remodeled into large and small particles. In addition, the reasons why remodeling is enhanced in TG-enriched HDL are not understood. The present study was undertaken in order to address these issues. The results show that the PLTP-mediated remodeling of recombinant HDL (rHDL) containing cholesteryl esters as the sole core lipid (CE-rHDL) and rHDL that are enriched with TG (TG-rHDL) involves the formation of a large, unstable fusion product, which either (i) rearranges into small particles in a process that is not accompanied by the dissociation of apoA-I or (ii) loses two molecules of apoA-I to form a more stable, large conversion product. The results also show that these processes occur independently and that the enhanced PLTP-mediated conversion of TG-rHDL into large and small particles may be due to the destabilization of apoA-I.

EXPERIMENTAL PROCEDURES

Isolation of ApoA-I—HDL were isolated from pooled, autologously donated samples of human plasma (Gribbles Pathology, Adelaide, South Australia) by ultracentrifugation in the 1.07 < d < 1.21 g/ml density range (15). The HDL were depleted by standard techniques (16). The resulting apo-HDL were subjected to anion exchange chroma-
Phospholipid Transfer Studies—To study phospholipid transfers from vesicles to rHDL, \([^{14}C]\)POPC-labeled small unilamellar POPC vesicles were mixed with unlabeled CE-rHDL or unlabeled TG-rHDL and incubated at 37 °C for 1, 3, 5, 10, and 20 min in the presence of PLTP (24). The vesicles were precipitated with a MnCl\(_2\)/heparin solution, and the radioactivity was determined by liquid scintillation counting (Beckman LS 6000 TA Liquid Scintillation Systems, Beckman Instruments, Inc., Fullerton, CA). Precipitation of the vesicles with MnCl\(_2\)/heparin was quantitative, while more than 95% of the rHDL remained in solution.

To study phospholipid transfers from rHDL to vesicles, \([^{14}C]\)POPC-labeled CE-rHDL or \([^{14}C]\)POPC-labeled TG-rHDL were reacted with unlabeled small unilamellar POPC vesicles and PLTP. The \([^{14}C]\)POPC-labeled CE-rHDL were prepared by incubating unlabeled CE-rHDL (6.6 µmol of PL) and \([^{14}C]\)POPC-labeled small unilamellar POPC vesicles (0.66 µmol of PL) with PLTP at 37 °C for 3 h. The radioabeled CE-rHDL were isolated by ultracentrifugation and then either incubated at 37 °C for 20 min with Intraplil and TBS, or enriched with TG by incubation at 37 °C for 20 min with Intraplil and CETP. The resulting \([^{14}C]\)POPC-labeled CE-rHDL and \([^{14}C]\)POPC-labeled TG-rHDL were isolated by ultracentrifugation. PLTP-mediated phospholipid transfers from the radiolabeled rHDL to unlabeled, small unilamellar POPC vesicles were determined by incubation at 37 °C for 1, 3, 5, 10, and 20 min (24).

Transfer rates were calculated as the slope of the initial, linear section of plots of the percentage of phospholipid transferred between the rHDL and vesicles as a function of time.

Interaction of PLTP with HDL—The binding of PLTP to CE-rHDL and TG-rHDL was studied using surface plasmon resonance analysis on a BIAcore 2000 system. Rabbit anti-mouse Fe (RAMFc) was covalently attached to CM5 research grade sensor chips as described (29). Purified monoclonal antibodies were injected individually at 10 µl/min and captured at 25 °C by the RAMFc. The human apoA-I-specific antibodies AI-1.2 (120 µg/ml), AI-11 (5 µg/ml), and AI-137.1 (13.5 µg/ml) were individually used to capture CE-rHDL and TG-rHDL. The human PLTP-specific antibody, mAb 66 (100 µg/ml) was used to capture the PLTP. The analyte PLTP or rHDL was exposed to the immobilized rHDL or PLTP, respectively, at 25 °C with a buffer flow rate of 10 µl/min.

For the apoA-I-specific mAb studies, a typical experiment consisted of the generation of a single chip of immobilized RAMFc followed sequentially by exposure of each flow cell to the same concentration of antibody and rHDL (25 nm). Then, 40 µl of PLTP analyte (125–1000 nm) was injected, with each flow cell receiving a different analyte concentration.

Epitope Mapping of Anti-PLTP mAb 66—Epitope mapping was performed using a Peptide-Spot filter containing 140 peptides, each 13 amino acid residues long, with 11 amino acid overlaps (Jenri Bio Tools, GmbH, Berlin, Germany). The filter was first soaked in methanol for 10 min at room temperature and further treated with monoclonal antibody mAb 66 as described (30). The bound antibodies were detected by ECL (30). According to this analysis, mAb 66 reacts with the region of amino acids 225–235 of PLTP, ATSINLDMDFEF. The molecular model of PLTP (31) predicts that this peptide region, especially the sequence STSN, is in a turn on the surface of PLTP and well exposed for antigenic reactions.2

Structural Studies—Phospholipid acyl chain and head group packing order was determined by labeling rHDL with 1,6-diphenyl-1,3,5-hexatriene (DPH) and 1,4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene \(p\)-tolenesulfonate (TMA-DPH) (32, 33), respectively. Steady state fluorescence polarization of the DPH- and TMA-DPH-labeled rHDL was measured at 5 °C intervals from 5 to 50 °C using an excitation wavelength of 386 nm. The molar ratio of rHDL phospholipid/probe was 500:1, and the final phospholipid concentration was 0.5 mm.

Lipid-water interfacial hydration was assessed by labeling the rHDL with 8-nitro-1,3,5-hexatriene (8-NO3-DPH) incorporated at various sites. Uncorrected 390–600 nm emission spectra of the PRODAN-labeled rHDL were recorded using an excitation wavelength of 386 nm and excitation and emission band pass of 5 and 6 nm, respectively. The molar ratio of rHDL phospholipid/probe was 500:1, and the final phospholipid content was 0.5 mm.

2 G. Wohlfahrt, personal communication.
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centration was 0.5 mM. The ratio of the emission intensities at 440 and 490 nm was measured at 5 °C intervals from 5 to 50 °C.

The unfolding of apoA-I in CE-rHDL and TG-rHDL was assessed by incubation with 0–8 M guanidine hydrochloride (GdnHCl) (21, 35). The rHDL were added to aliquots of 50 mM Tris-HCl, pH 8.0, containing varying concentrations of GdnHCl. The final apoA-I concentration was 20 μg/ml. Wavelengths of maximum fluorescence were determined from 300–380-nm emission scans using an excitation wavelength of 295 nm. The respective excitation and emission band passes were 10 and 5 nm. Initial readings (t = 0 h) were made at 25 °C, immediately after the rHDL were added to the GdnHCl. Subsequent measurements were made after 2, 5, 8, and 24 h of incubation at 25 °C.

Other Methods—Nondenaturing 3–40% polyacrylamide gradient gel electrophoresis was used to quantitate rHDL size (36). The gels were stained with Coomassie Blue and scanned with a Sharp JX-610 scanner (Sharp, Japan). Image master software was used to quantitate particle size by reference to high molecular weight standards of known diameter (Amersham Pharmacia Biotech). Agarose gel electrophoresis was used to determine rHDL surface charge (37).

A COBAS FARA centrifugal analyzer (Roche Diagnostics, Zurich, Switzerland) was used for the compositional analyses. ApoA-I concentrations were determined either by the method of Lowry et al. (38), using bovine serum albumin as a standard, or by an immunoturbidometric assay (39). Enzymatic kits (Roche Molecular Biochemicals) were used to measure phospholipid, UC, and total cholesterol concentrations. CE concentrations were calculated as the difference between the total cholesterol and UC concentrations. TG was quantitated enzymatically (40).

The number of apoA-I molecules/particle was determined by covalently cross-linking the rHDL with bis(sulfosuccinimidyl) suberate (41). The cross-linked rHDL and a control sample of cross-linked, lipid-free apoA-I were electrophoresed on nondenaturing polyacrylamide gradient gels. The number of apoA-I molecules/particle was determined by reference to the cross linked lipid-free apoA-I.

Statistical Analysis—Analysis of variance, two-factor with repeated measures, was used to assess differences between data sets. Significance was determined as p < 0.05. The data analysis package in Microsoft Excel 98 was used for these analyses.

RESULTS

Remodeling of CE-rHDL and TG-rHDL by PLTP (Tables I and II, Figs. 1–3)—Spherical CE-rHDL, prepared as described under “Experimental Procedures,” were enriched with TG by incubation for 20 min with Intralipid and CETP. The respective diameters of the CE-rHDL and TG-rHDL were 9.3 and 9.5 nm, and their PL/UC/CE/TG/apoA-I molar ratios were 95.3/6.8/68.1/1.8/3.0 and 103.2/6.2/54.2/22.9/3.0 (Table I). The small amount of TG in the CE-rHDL probably represents traces of Intralipid co-isolating with the rHDL rather than spontaneous transfer of TG from Intralipid to the rHDL (21). Since cross-linking with bis(sulfosuccinimidyl) suberate showed that the CE-rHDL and TG-rHDL both contained three molecules of apoA-I/particle, the stoichiometries are expressed relative to three molecules of apoA-I. As judged by agarose gel electrophoresis, TG enrichment did not affect rHDL surface charge, with the CE-rHDL and TG-rHDL both having electrophoretic mobilities of −0.49 μm s −1 V −1 cm −1.

CE-rHDL and TG-rHDL size was not affected by incubation for 24 h in the absence of PLTP (Fig. 1). After 24 h of incubation in the presence of PLTP, ~76% of the original CE-rHDL were converted into large (11.3-nm) and small (7.7-nm) particles. The remaining CE-rHDL were unchanged in size. When the TG-rHDL were incubated for 24 h with PLTP, they were completely converted into large (11.3-nm) and small (7.7-nm) particles, thus confirming the earlier report that TG-enrichment enhances the remodeling of HDL by PLTP (9).

The large and small conversion products were resolved by gel permeation chromatography (Fig. 2). The lipid and protein constituents in the rHDL that were either maintained at 4 °C or incubated at 37 °C in the absence of PLTP eluted as a single peak between fractions 12 and 27. This reflects the monodispersity of the rHDL preparations. The large particles generated by incubation in the presence of PLTP were enriched in CE, while the small conversion products were enriched in apoA-I. Selected fractions were pooled as indicated (→) and subjected to nondenaturing gradient gel electrophoresis (Fig. 3). Profiles A and B show, respectively, the CE-rHDL and TG-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 represent the large (11.3-nm) and small (7.7-nm) conversion products, respectively. The number of apoA-I molecules/particle in the pooled samples was determined by cross-linking (Table II). Whereas the original CE-rHDL and TG-rHDL contained three molecules of apoA-I/particle, large and small conversion products contained four and two molecules of apoA-I/particle, respectively. The composition of the pooled samples and the recoveries of the individual rHDL constituents are shown in Table II. The large particles contained approximately twice as many phospholipid and core lipid molecules as the original rHDL, indicating that they are derived from a fusion product. Following incubation with PLTP, the recovery of rHDL core lipids exceeded that of the surface constituents. This indicates

### Table I

| rHDL        | Stoichiometry | Stokes diameters | Number of apoA-I molecules/particle | Electrophoretic mobility |
|-------------|---------------|-----------------|-------------------------------------|--------------------------|
| CE-rHDL     | PL/UC/CE/TG/apoA-I | 9.3             | 3                                   | −0.49                    |
| TG-rHDL     |               | 9.5             | 3                                   | −0.49                    |

* Determined by nondenaturing 3–40% polyacrylamide gradient gel electrophoresis.

* Determined by cross-linking.

* Determined by agarose gel electrophoresis.

![Fig. 1. PLTP-mediated changes in rHDL size.](http://www.jbc.org/)

**Physical properties of CE-rHDL and TG-rHDL**

Spherical CE-rHDL (final CE concentration 0.1 mM/liter) were incubated with Intralipid (final TG concentration 4.0 mM/liter) in the absence and presence of CETP (final activity 2.7 units/ml) at 37 °C for 20 min. The final volume of the incubation mixture was 68.0 ml. When the incubations were complete, the rHDL were isolated by ultracentrifugation. Stoichiometries were determined as the mean of triplicate determinations, which varied by less than 10%.

![Fig. 2.](http://www.jbc.org/)
that phospholipids and UC as well as apoA-I dissociated from the CE-rHDL and TG-rHDL during the incubation.

Time Course of the Remodeling of CE-rHDL and TG-rHDL by PLTP (Fig. 4)—The time dependence of the remodeling of CE-rHDL and TG-rHDL by PLTP was also examined (Fig. 4). CE-rHDL and TG-rHDL were incubated for 0–24 h with PLTP. Aliquots of the incubation mixtures were subjected to nondenaturing polyacrylamide gel electrophoresis. Scans of the Coomassie-stained gels are shown. Profiles A and B, respectively, show the pooled CE-rHDL and TG-rHDL fractions that were either maintained at 4 °C or incubated at 37 °C for 24 h in the absence of PLTP. Profiles C and D are the large and small conversion products generated by incubation of CE-rHDL and TG-rHDL with PLTP at 37 °C for 24 h.

The finding that the small conversion products were formed before the large conversion products provides convincing evidence that these particles are generated by two independent processes. Furthermore, as both the large and small TG-rHDL conversion products appeared more rapidly than the CE-rHDL conversion products, it follows that TG enrichment enhances both processes. The additional finding that the dissociation of apoA-I coincided with the appearance of the large conversion products suggests that their formation is related.

Influence of TG Enrichment on Phospholipid Transfers (Fig. 5)—To determine whether transfers of phospholipids from small unilamellar vesicles to TG-HDL were also enhanced relative to CE-rHDL, the rHDL were incubated with [14C]POPC-labeled small unilamellar POPC vesicles and PLTP for 0–20 min. Transfers of [14C]POPC from the vesicles to CE-rHDL (open squares) and TG-rHDL (closed squares) are shown in Fig. 5. The initial rate of transfer from the vesicles to TG-rHDL was 7.1 μmol of POPC/ml of PLTP/h, compared with 3.9 μmol of POPC/ml of PLTP/h for CE-rHDL.

To determine whether the different rates of transfer could be explained by modification of the vesicles by rHDL surface constituents, transfers of phospholipids from rHDL to the vesicles were also examined. [14C]POPC-labeled CE-rHDL and [14C]POPC-labeled TG-rHDL were incubated for 0–20 min with PLTP and unlabeled POPC vesicles. There was no detectable transfer of POPC from the CE-rHDL to the vesicles (open diamonds). Less than 5% of the TG-rHDL phospholipids transferred to the vesicles (closed diamonds). This indicates that the different rates of transfer of phospholipids from the vesicles to the rHDL cannot be explained by rHDL phospholipids altering the vesicle structure.

Binding of CE-rHDL and TG-rHDL to PLTP (Table III)—There was no difference between the interaction of soluble PLTP with CE-rHDL and TG-rHDL that were immobilized on the biosensor surface using either of three separate apoA-I-specific monoclonal antibodies (AI-1.2, AI-11, or AI-137.1). Likewise, no difference was observed between the interaction of CE-rHDL and TG-rHDL with PLTP when the PLTP was immobilized on the biosensor surface with the PLTP-specific antibody mAb 66 (Table III).

The Influence of TG Enrichment on rHDL Structure (Table IV, Fig. 6)—Since the enhanced remodeling of TG-rHDL compared with CE-rHDL could not be explained in terms of differences in the binding to PLTP, the possibility that it was due to structural differences between the particles was investigated.

Spherical CE-rHDL were enriched with increasing amounts of TG by incubation with Intralipid and CETP for 2 or 20 min.
Remodeling of HDL by PLTP

**TABLE II**

Physical properties of the pooled rHDL fractions

| Incubation conditions | Stoichiometry | Recoverya | Stokes diameterb | Number apoA-I molecules/particlec |
|-----------------------|---------------|------------|------------------|-----------------------------------|
|                       | PL | UC | CE | TG | A-I | PL | UC | CE | TG | A-I | nm |                                |
| CE-rHDL               | 73.5 | 7.0 | 71.7 | 2.0 | 3.0 | 100 | 100 | 100 | 100 | 100 | 9.3 | 3 |
| -PLTP, 4 °C           | 78.3 | 6.9 | 72.6 | 2.0 | 3.0 | 99  | 105 | 98  | 103 | 101 | 9.3 | 3 |
| +PLTP, 37 °C          | 148.3 | 10.8 | 157.7 | 4.2 | 4.0 | 36  | 23  | 48  | 42  | 29  | 11.3 | 4 |
| Large conversion products | 48.8 | 3.0 | 44.8 | 1.5 | 2.0 | 29  | 22  | 34  | 39  | 35  | 7.7 | 2 |
| Small conversion products | (65) | (45) | (82) | (64) |      |      |      |      |      |      |      |    |
| TG-rHDL               | 76.8 | 6.0 | 72.9 | 21.3 | 3.0 | 100 | 100 | 100 | 100 | 100 | 9.5 | 3 |
| -PLTP, 4 °C           | 71.1 | 6.9 | 75.0 | 22.5 | 3.0 | 99  | 109 | 95  | 101 | 94  | 9.5 | 3 |
| +PLTP, 37 °C          | 143.6 | 12.0 | 153.5 | 50.8 | 4.0 | 27  | 11  | 44  | 35  | 33  | 11.3 | 4 |
| Large conversion products | 54.6 | 3.6 | 52.6 | 16.0 | 2.0 | 23  | 11  | 28  | 35  | 33  | 7.7 | 2 |
| Small conversion products | (50) | (22) | (72) | (70) | (53) |    |    |    |    |    |    |    |

a Recoveries of individual constituents are expressed relative to the total recovery of constituents in the large and small conversion products. Values in parenthesis represent the mean of triplicate determinations, which varied by less than 10%.
b Determined by nondenaturing polyacrylamide gradient gel electrophoresis.
c Determined by cross-linking.

Control incubations containing CE-rHDL and Intralipid but no CETP were also carried out. The rHDL that were incubated with CETP contained TG as 12.2 and 32.4% of the total core lipids (Table IV). TG enrichment increased the size of the rHDL slightly from 9.2 to 9.5 nm.

Phospholipid acyl chain and head group packing order was determined from the steady state fluorescence polarization of rHDL labeled with DPH and TMA-DPH (Fig. 6). The DPH-labeled CE-rHDL that were either maintained at 4 °C (data not shown) or incubated in the absence of CETP (closed squares) had comparable polarization values. As the TG content of the rHDL increased, phospholipid acyl chain packing order decreased as evidenced by the progressive reduction in the polarization values for the rHDL with 12.2% TG (open triangles) (p < 0.001 compared with all other samples by analysis of variance) and 32.4% TG (open circles) (p < 0.001 compared with all other samples by analysis of variance).

The polarization values for the TMA-DPH-labeled CE-rHDL (closed squares) and the TG-rHDL with 12.2% TG (open triangles) and 32.4% TG (open circles) were not significantly different, indicating that TG enrichment did not affect rHDL phospholipid head group packing order.

The rHDL lipid-water interfacial hydration was assessed by labeling with PRODAN and comparing the ratio of the intensities of the fluorescence emission spectra at 440 and 490 nm as a function of temperature. No differences were observed between the CE-rHDL and TG-rHDL (results not shown). This indicates that TG enrichment has no effect on rHDL lipid-water interfacial hydration.

The Influence of TG Enrichment on the Unfolding of apoA-I in rHDL (Table IV, Figs. 7 and 8)—The unfolding of the apoA-I in spherical CE-rHDL and rHDL containing either 12.2 or 32.4% TG is shown in Fig. 7. The rHDL were incubated with 0–8 M GdnHCl for 0 (closed diamonds), 5 (open squares), and...
The association rate constants ($k_a$) and dissociation rate constants ($k_d$) for PLTP and CE-rHDL or TG-rHDL were measured by surface plasmon resonance analysis as described under “Experimental Procedures.” Multiple interactions were studied including the interaction of soluble PLTP with rHDL that had been immobilized on the biosensor chip with three unique apoA-I-specific monoclonal antibodies. The interaction of soluble rHDL with PLTP immobilized with the PLTP-specific monoclonal antibody 66 was also studied. Monoclonal antibodies AI-11, AI-137, and AI-1.2 bind apoA-I epitopes containing amino acid residues 96–111, 137–147, and 1–19, respectively.

Table III

| mAb         | CE-rHDL $k_a$ | TG-rHDL $k_a$ | CE-rHDL $K_d$ | TG-rHDL $K_d$ |
|-------------|--------------|---------------|---------------|---------------|
| AI-11       | 3.65 x 10^2  | 2.81 x 10^2   | 2.75 x 10^3   | 2.04 x 10^4   |
| AI-137.1    | 6.88 x 10^2  | 5.02 x 10^2   | 1.19 x 10^3   | 3.94 x 10^4   |
| AI-1.2      | 7.21 x 10^2  | 4.38 x 10^2   | 1.68 x 10^3   | 2.62 x 10^4   |
| Anti-PLTP   | 1.54 x 10^2  | 3.32 x 10^2   | 1.65 x 10^3   | 1.70 x 10^4   |

Table IV

Unfolding of apoA-I in CE-rHDL and TG-rHDL

Spherical CE-rHDL (final CE concentration 0.1 mM/liter) were mixed with Intralipid (final concentration 4.0 mM/liter) and either maintained at 4 °C or incubated at 37 °C for 20 min in the absence of CETP or incubated at 37 °C for 2 and 20 min in the presence of CETP (2.7 units/ml of incubation mixture). The final volume of the incubation mixtures was 5.0 ml. When the incubations were complete, the rHDL were isolated by ultracentrifugation. The stoichiometries represent the mean of triplicate determinations, which varied by less than 10%.

Table IV

| Incubation conditions | Stoichiometry | mol of TG | Stokes diameter | [GdnHCl]_{1/2} | [GdnHCl]_{1/2} |
|-----------------------|---------------|-----------|-----------------|----------------|----------------|
| + Intralipid – CETP, 4 °C, 20 min | 84.6/5.7/90.7/6.3/0 | 1.0 | 9.2 | ND | ND |
| + Intralipid – CETP, 37 °C, 20 min | 88.5/5.1/81.8/1.8/3.0 | 2.8 | 9.2 | 3.5 ± 0.13 | 3.2 ± 0.02 |
| + Intralipid + CETP, 37 °C, 2 min | 91.8/6.0/58.8/1.3/0 | 12.2 | 9.3 | 3.2 ± 0.08 | 3.0 ± 0.04 |
| + Intralipid + CETP, 37 °C, 20 min | 103.2/6.7/43.9/21.0/3.0 | 32.4 | 9.5 | 2.5 ± 0.09 | 2.5 ± 0.03 |

a Determined by nondenaturing polyacrylamide gradient gel electrophoresis.

b Concentration of GdnHCl required to achieve 50% unfolding of apoA-I. Determined directly from Fig. 7.

c Concentration of GdnHCl required to achieve 50% unfolding of apoA-I. Calculated as described (35).
d ND, not determined.

24 h (closed triangles). The results for the CE-rHDL are shown in Fig. 7A. Panels B and C represent rHDL with 12.2 and 32.4% TG, respectively. The concentration of GdnHCl required to achieve 50% unfolding of apoA-I is shown in Table IV. These values were either calculated directly from Fig. 7, or from a plot of the concentration of GdnHCl versus the free energy of unfolding of apoA-I (35). The results show that the concentration of GdnHCl required to unfold apoA-I decreases with increasing rHDL TG content.

The kinetics of the unfolding of apoA-I in CE-rHDL (closed diamonds) and rHDL with 12.2% (open squares) and 32.4% (closed triangles) TG are shown in Fig. 8. These experiments were carried out in the presence of 4.0 mM GdnHCl. The apoA-I in the rHDL with 32.4% TG unfolded more rapidly than the apoA-I in TG-rHDL. The unfolding of apoA-I in CE-rHDL and TG-rHDL was measured by incubating CE-rHDL and TG-rHDL with 32.4% TG by nondenaturing polyacrylamide gradient gel electrophoresis.

Discussion

The ability of PLTP to remodel HDL into large and small particles, mediate the dissociation of apoA-I from HDL, and transfer phospholipids between HDL and other lipoproteins is well documented (1–8). Earlier work from this laboratory has also established that TG enrichment enhances the remodeling of HDL by PLTP (9). While the PLTP-mediated remodeling of HDL and the dissociation of apoA-I are both dependent on efficient phospholipid transfers (42), the mechanism by which these processes occur is not understood. The present study was carried out in order to elucidate the mechanism of the remodeling of HDL by PLTP and reasons why it is enhanced in TG-enriched particles.

These goals were achieved by (i) characterizing the large and small particles that were formed when CE-rHDL and TG-rHDL were incubated with PLTP, (ii) determining the time sequence for the formation of the large and small particles and the dissociation of apoA-I, and (iii) defining the structural changes that occur when rHDL are enriched with TG.
with PLTP and monitoring rHDL size changes as well as the dissociation of apoA-I (Fig. 4). These results showed that the small conversion products were formed before the large conversion products and that the appearance of the large conversion products coincided with the dissociation of apoA-I. Furthermore, both the large and small TG-rHDL conversion products were formed more rapidly than the CE-rHDL conversion products.

In the case of CE-rHDL, small particles were formed during the first 6 h of incubation with PLTP, while the large conversion products and dissociated apoA-I appeared after 12 h of incubation. These time differences indicate that the large and small conversion products are formed by independent processes. Furthermore, the finding that the formation of the large particles coincided with the dissociation of apoA-I suggested that both of these species were generated via a common pathway.

The data in Table II show that the large CE-rHDL and TG-rHDL conversion products contain more surface and core lipid constituents/particle than the original rHDL, indicating that they must have been formed by particle fusion. Moreover, the data in Table III, showing that TG enrichment does not affect the binding of PLTP to rHDL, suggests that the rate at which the rHDL are remodeled is determined by processes that occur after PLTP has mediated particle fusion. This finding, together with the data in Table II and Fig. 4, indicate that the PLTP-mediated remodeling of rHDL involves the following events (Fig. 9).

The possibility that the small conversion products are further remodeled into large conversion products was also considered. The data in Table II indicate that this pathway requires interactions between three small conversion products and the concomitant dissociation of two molecules of apoA-I. Although the results in Fig. 4 indicate that formation of large conversion products by this pathway is feasible, it is likely that trimolecular collisions of this type are not energetically favorable. As
such, this pathway is unlikely to be a major source of the large conversion products.

The results of the spectroscopic studies give an insight as to why the TG-rHDL are remodeled by PLTP more rapidly than CE-rHDL. The data in Fig. 8 show that the apoA-I in TG-rHDL unfolds more readily than apoA-I in CE-rHDL. This is in agreement with what has been reported by other investigators (43) and is most likely caused by TG partitioning from the core into the particle surface and preventing apoA-I α-helices from intercalating between the rHDL phospholipid acyl chains (44). This may be why apoA-I dissociates more rapidly from TG-rHDL than from CE-rHDL and why the PLTP-mediated remodeling of HDL is enhanced by TG enrichment.

The apoA-I that dissociated from the CE-rHDL and TG-rHDL during the incubations with PLTP appeared as three bands when subjected to nondenaturing gradient gel electrophoresis and immunoblotting (Fig. 4). One of the bands was comparable in size with lipid-free apoA-I. The smallest band is possibly the 23-kDa fragment of apoA-I that is generated when either lipid-free or lipid-associated apoA-I are incubated with PLTP (45). The largest of the three bands may represent apoA-I complexed with small amounts of HDL lipids. This is consistent with the reduced recovery of surface constituents relative to core lipids in the large and small conversion products (Table II). It is possible that the apoA-I that is associated with small amounts of lipid may be comparable with the pre-β2-migrating HDL in human plasma that have been identified as the initial acceptors of cellular cholesterol from peripheral tissues (12, 46).

Fig. 5 shows that PLTP-mediated phospholipid transfers from small unilamellar vesicles to TG-rHDL are enhanced in TG-rHDL relative to CE-rHDL. The additional finding that there is minimal transfer of phospholipids in the reverse direction, from the rHDL to the vesicles, excludes the possibility that this result can be explained by rHDL phospholipids altering the vesicle surface. The increased transfer of phospholipids from the vesicles to the TG-rHDL can be explained by an enhanced ability of the TG-rHDL to accommodate additional phospholipids. This is consistent with the results in Fig. 6, which show that TG enrichment decreases the packing order of HDL phospholipid acyl chains. This is probably a result of TG partitioning from the core into the rHDL surface and generating packing defects that can be occupied by additional phospholipid molecules. These findings are also in agreement with the observation that the TG content of HDL correlates positively with the rate of PLTP-mediated phospholipid transfers in plasma (47).

In conclusion, this study provides the first insight into the mechanism by which PLTP mediates the remodeling of HDL. The results show that PLTP acts as a fusogen when it interacts with rHDL and that the fusion product is subsequently remodeled into large and small particles. Evidence that the large and small conversion products are formed by two independent processes is also presented. In addition, we show that the apoA-I in TG-rHDL is destabilized compared with the apoA-I in CE-rHDL and that this destabilization may be responsible for the enhanced PLTP-mediated remodeling of TG-rHDL.

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REFERENCES

1. Tollefsen, J. H., Ravnik, S., and Albers, J. J. (1988) J. Lipid Res. 29, 1593–1602
2. Rao, R., Albers, J. J., Wolfbauer, G., and Pownall, H. J. (1997) Biochemistry 36, 3645–3653
3. Jauhiainen, M., Metso, J., Pahlman, R., Blomqvist, S., van Tol, A., and Ehnholm, C. (1993) J. Biol. Chem. 268, 4032–4036
4. Tu, A. Y., Nishida, H. I., and Nishida, T. (1993) J. Biol. Chem. 268, 23098–23105
5. Albers, J. J., Wolfbauer, G., Cheung, M. C., Day, J. R., Ching, A. F. T., and Tu, A. Y. (1995) Biochim. Biophys. Acta 1258, 27–34
6. Pusininen, S., Jauhiainen, M., Metso, J., Tynnela, J., and Ehnholm, C. (1995) J. Lipid Res. 36, 975–985
7. Lusas, S., Jauhiainen, M., Metso, J., Somerharju, P., and Ehnholm, C. (1996) Biochim. Biophys. Acta 1253, 275–282
8. Korhonen, A., Jauhiainen, M., Pahlman, R., and Albers, J. J. (1997) Biochim. Biophys. Acta 1339, 103–110
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