Active plasma phospholipid transfer protein is associated with apoA-I- but not apoE-containing lipoproteins

Marian C. Cheung[1] and John J. Albers

Division of Metabolism, Endocrinology, and Nutrition, Department of Medicine, School of Medicine, University of Washington, Seattle, WA

Abstract **Plasma phospholipid transfer protein (PLTP) is a multifaceted protein with diverse biological functions. It has been shown to exist in both active and inactive forms. To determine the nature of lipoproteins associated with active PLTP, plasma samples were adsorbed with anti-A-I, anti-A-II, or anti-E immunoadsorbent, and PLTP activity was measured in the resulting plasma devoid of apolipoprotein A-I (apoA-I), apoA-II, or apoE. Anti-A-I and anti-A-II immunoadsorbents removed 98 ± 1% (n = 8) and 38 ± 25% (n = 7) of plasma PLTP activity, respectively. In contrast, only 1 ± 5% of plasma PLTP activity was removed by anti-E immunoadsorbent (n = 7). Dextran sulfate (DS) cellulose did not bind apoA-I, but it removed 83 ± 5% (n = 4) of the PLTP activity in plasma. In size-exclusion chromatography, PLTP activity removed by anti-A-I or anti-A-II immunoadsorbent was associated primarily with particles of a size corresponding to HDL, whereas a substantial portion of the PLTP activity dissociated from DS cellulose was found in particles larger or smaller than HDL. These data show the following: *1*) active plasma PLTP is associated primarily with apoA-I- but not apoE-containing lipoproteins; *2*) active PLTP is present in HDL particles with and without apoA-II, and its distribution between these two HDL subpopulations varies widely among individuals; and *3*) DS cellulose can remove active PLTP from apoA-I-containing lipoproteins, and this process creates new active PLTP-containing particles in vitro.**—Cheung, M. C., and J. J. Albers. **Active plasma phospholipid transfer protein is associated with apoA-I- but not apoE-containing lipoproteins. *J. Lipid Res.* 2006.** 47: **1315–1321.**

Supplementary key words apolipoprotein A-I • apolipoprotein A-II • apolipoprotein E • dextran sulfate cellulose

Plasma phospholipid transfer protein (PLTP) plays multiple roles in lipoprotein metabolism. It can facilitate the transfer of surface lipids from triglyceride-rich lipoproteins to HDL during lipolysis (1–3) and can convert HDL into larger and smaller particles, generating preβ HDL in the process (4, 5). PLTP also interacts with the ATP binding cassette transporter A1 and enhances lipid efflux from cells (6, 7). Besides its roles in lipid metabolism, PLTP can bind and transfer lipopolysaccharide (8) and α-tocopherol (9) between lipoproteins and cells, and it may modify the antioxidative potentials of lipoproteins and tissues (10).

Two forms of PLTP have been shown to exist in plasma. The "active" form has the ability to transfer phosphatidylcholine from phospholipid vesicles to HDL in an assay originally developed by Damen, Regts, and Scherphof (11), and the "inactive" form has little or no ability to transfer phospholipids under similar conditions (12, 13). These two forms of PLTP are associated with lipoproteins with different characteristics. Inactive PLTP is located between LDL and HDL in size-exclusion chromatography, having an average apparent molecular mass of 520 kDa (12) and a Stokes diameter of 12 to >17 nm (14). Active PLTP, in contrast, is associated with smaller lipoproteins, with an average molecular mass of 160 kDa and a Stokes diameter between 7.6 and 12.0 nm (12, 14). Lipoproteins containing active PLTP were reported to be associated with apolipoprotein E (apoE) but not apoA-I, whereas inactive PLTP was found to be associated with apoA-I but not apoE (13). Recently, apoE and apoA-IV proteoliposomes have been reported to activate inactive PLTP in vitro (15). Based on these findings, it has been hypothesized that PLTP is secreted as a high-activity complex, and as it becomes complexed to the apoA-I-containing particles during the transfer of surface phospholipids from triglyceride-rich lipoproteins to HDL, it loses its ability to transfer phospholipids (13, 15).

To understand the molecular differences between active and inactive PLTP in human plasma, and their physiological relevance, it is essential to know the nature of the lipoproteins associated with these forms of PLTP. Because active and inactive PLTP have been found to be associated selectively with apoE and apoA-I, respectively (13), we reasoned that plasma samples devoid of apoA-I (i.e., with inactive PLTP removed) would be a good starting material for isolating active PLTP complexes, and plasma samples

Manuscript received 27 January 2006 and in revised form 3 March 2006.

Published, JLR Papers in Press, March 6, 2006.
DOI 10.1194/jlr.M600042-JLR200

[1] To whom correspondence should be addressed.
e-mail: mccheung@u.washington.edu

devoid of apoE (i.e., with active PLTP removed) would be a good starting material for isolating inactive PLTP complexes. However, we found that our anti-A-I immunoadsorbent removed essentially all plasma PLTP activity, whereas our anti-E immunoadsorbent removed negligible amounts of PLTP activity. Because these results directly contradicted existing reports on the distribution of the active form of PLTP among lipoproteins and may be of significance in formulating future studies related to these two forms of PLTP in human plasma, we report here our findings on the distribution of active PLTP among apoA-I-, apoA-II-, and apoE-containing lipoproteins and provide a possible explanation for the divergent findings among laboratories.

METHODS

Blood samples

Blood samples used in this study were obtained from adult individuals after a 12–14 h overnight fast, and informed consent was obtained. Venous blood was drawn into Vacutainer tubes (Becton-Dickinson) containing disodium EDTA. Upon separation of plasma at 4°C by low-speed centrifugation, sodium azide, chloramphenicol, and gentamycin were promptly added to final concentrations of 0.1, 0.01, and 0.005 g/l, respectively, and the resulting material was used for immunoadsorption studies or for lipoprotein isolation.

Preparation of immunoadsorbents

Antibodies against human apoA-I, apoA-II, and apoE were produced by immunizing goats with the purified apolipoproteins. Specific goat anti-A-I and anti-A-II antibodies were isolated from the respective antiserum by affinity chromatography using HDL covalently coupled to cyanogen bromide-activated Sepharose 4B (Amersham Pharmacia Biotech), as described (16). Anti-E antibodies were similarly isolated using affinity Sepharose 4B gels containing lipoproteins of density < 1.019 g/ml. These antibodies were tested to be specific for the respective antigen by immunoprecipitation and/or immunoblotting. Immunoprecipitation was performed with ^{125}I-labeled apoA-I, apoA-II, apoC-I, apoC-II, apoC-III, apoD, apoE, LDL, and LCAT, as described (16). Immunoblotting was performed according to the method of Towbin, Staehelin, and Gordon (17) using VLDL, HDL, apoA-I, apoA-II, and/or apoE as the testing antigens. Affinity-isolated antibodies were coupled to cyanogen bromide-activated-Sepharose 4B at 4–8 mg/ml according to the manufacturer's procedure to generate anti-A-I, anti-A-II, and anti-E immunoadsorbents. The binding capacities of these immunoadsorbents were approximately 200 μg of apoA-I, 50 μg of apoA-II, and 25 μg of apoE per milliliter of gel. All affinity gels were stored in 0.01 M Tris buffer, pH 7.4, containing 0.15 M NaCl, 1 mM EDTA, and 0.01% sodium azide (Tris buffer).

Immunoadsorption studies

Forty milliliters each of anti-A-I and anti-A-II immunoadsorbent, and 20 ml of anti-E immunoadsorbent, were packed into separate Kontes Flex columns of 2.5 cm internal diameter. Plasma samples (4–6 ml) were loaded into the immunoadsorbents at 10–12 ml/h, and proteins that did not bind to the antibodies were washed extensively with Tris buffer at the same flow rate overnight and collected in 6 ml fractions. All fractions with absorption readings at 280 nm of >0.1 were combined and concentrated under vacuum to within 1 ml of the starting plasma volume using Micro-ProDiCon membranes (Molecular weight cut-off = 10,000; Spectrum) for apoA-I, apoA-II, or apoE measurements to confirm their absence and for PLTP activity determination. To recycle the immunoadsorbents, bound lipoproteins were dissociated with 3 M sodium thiocyanate in 0.02 M sodium phosphate, pH 7.0 (NaSCN). These lipoproteins were either discarded or desalted and concentrated for further studies. All procedures were performed at 4°C.

Isolation of HDL particles by immunoaffinity chromatography

Human HDL is composed of two major populations of particles. One population contains both apoA-I and apoA-II [Lp(A-I,A-II)], and the other contains apoA-I but not apoA-II [Lp(A-I)]. We routinely isolate these HDL particles from plasma using an established sequential anti-A-II and anti-A-I affinity column chromatography procedure (16) with the addition of a dextran sulfate (DS) cellulose column preceding the antibody columns. The DS cellulose (Kanegafuchi Chemical) binds apoB-containing lipoproteins but not apoA-I- or apoA-II-containing lipoproteins (18, 19). It allows the removal of VLDL and LDL from HDL particles. The apoB-containing lipoproteins, Lp(A-I,A-II), and Lp(A-I) bound to DS, anti-A-II, and anti-A-I columns, respectively, were rapidly desorbed with NaSCN and immediately desalted with Sephadex G25 (Amersham Pharmacia Biotech). The essentially lipoprotein-free flow-through plasma and the eluted lipoproteins were concentrated with Micro-ProDiCon membranes as described above to volumes less than or equal to the starting plasma for apolipoprotein and PLTP activity measurements.

In some experiments, the DS column was omitted to determine the effect of DS on the association of active PLTP with lipoproteins. In these isolations, apoB-containing lipoproteins remained in the flow-through apoA-I-deficient plasma.

Size-exclusion chromatography

Plasma or lipoprotein samples for fast-protein liquid chromatography (FPLC) size-exclusion chromatography were filtered through 0.45 μm membranes, and 0.2–0.5 ml of each sample was applied to a Superose 6 HR 10/30 column (10 × 300 mm; Amersham Pharmacia Biotech) at 4°C and eluted at a flow rate of 12 ml/h with 0.05 M sodium phosphate buffer, pH 7.4, containing 0.15 M NaCl, 0.01% EDTA, and 0.02% sodium azide. Sixty fractions of 0.5 ml each were collected for PLTP activity and cholesterol measurement.

Distribution of PLTP activity

Phospholipid transfer activity mediated by PLTP was determined by measuring the transfer of [^{14}C]phosphatidylcholine from phospholipid liposomes to HDL using an established radioassay (20). To assess the proportion of PLTP associated with apoA-I, apoA-II, and apoE, PLTP activity levels in whole plasma, in plasma devoid of apoA-I, apoA-II, or apoE, and in affinity-isolated lipoproteins were measured under the conditions in which the percentage of phospholipid transfer was directly proportional to the amount of the test samples (i.e., the linear range of the assay).

To account for losses in sample handling in postadsorbed plasma, the protein or albumin level in plasma and postadsorbed plasma was measured. The activity measured in the postadsorbed plasma after adjustment for protein or albumin recovery was considered to be the amount of PLTP activity in postadsorbed plasma. Protein and albumin recovery in the plasma

after adsorption with anti-A-I, anti-A-II, and anti-E were 90 ± 9% (n = 8), 88 ± 9% (n = 7), and 96 ± 9% (n = 7), respectively.

To account for losses in sample handling in affinity-isolated lipoproteins, apoA-I, apoA-II, and apoB were measured in plasma and in the lipoproteins. The activity detected in the lipoproteins after adjustment of apoA-I, apoA-II, or apoB recovery was considered to be the amount of PLTP activity associated with Lp(A-I), Lp(A-I,A-II), or the apoB-containing lipoproteins bound to DS cellulose, respectively. Recovery of apoA-I, apoA-II, and apoB eluted from the anti-A-I, anti-A-II, and DS columns was 87 ± 9%, 88 ± 9%, and 90 ± 6%, respectively, for 22 isolations. Total PLTP activity recovered from these columns after appropriate apolipoprotein adjustment was 51 ± 17%. The low recovery in PLTP activity indicates partial inactivation of PLTP by NaSCN, as reported previously for cholesteryl ester transfer protein (CETP) and LCAT (21).

Other analytical procedures

PLTP mass in isolated lipoproteins was examined by immunoblotting after separation of lipoproteins on a reducing SDS 4–15% gradient gel (Bio-Rad). Proteins on the gel were transferred to a 0.2 μm nitrocellulose membrane according to the method of Towbin, Staehelin, and Gordon (17). PLTP was identified using a rabbit anti-PLTP antibody prepared in our laboratory (14) as primary antibody, horseradish peroxidase-conjugated goat anti-rabbit IgG (Kirkegaard and Perry) as secondary antibody, and chemiluminescent substrates (Pierce) as detecting agents. ApoA-I, apoA-II, apoB, apoE, and albumin concentrations were measured with nephelometric assays using reagents from Dade Behring Diagnostics. Cholesterol was measured using enzymatic methods. Apolipoproteins and cholesterol analyses were performed at the Northwest Lipid Research Laboratories.

RESULTS

PLTP activity in plasma devoid of apoA-I, apoA-II, or apoE

When eight plasma samples were adsorbed with anti-A-I immunoadsorbent, only 2 ± 1% of the original plasma PLTP activity was detected in the postadsorbed plasma devoid of measurable apoA-I (**Table 1**). Thus, 98 ± 1% of plasma PLTP activity was associated with apoA-I-containing lipoproteins. However, when separate aliquots of seven of the eight plasma samples were adsorbed with anti-A-II immunoadsorbent, 62 ± 25% of PLTP activity was detected in the apoA-II-deficient plasma (Table 1). Because anti-A-I immunoadsorbent removed PLTP activity associated with both Lp(A-I) and Lp(A-I,A-II), whereas anti-A-II immunoadsorbent removed only the PLTP activity associated with Lp(A-I,A-II), by difference, 60 ± 25% of plasma PLTP activity was associated with Lp(A-I) and 38 ± 25% of plasma PLTP activity was associated with Lp(A-I,A-II) in these plasma samples. Although on average, more active PLTP was associated with HDL particles without apoA-II than with apoA-II, the distribution of PLTP activity between these two HDL subpopulations varied considerably among the plasma samples studied, ranging from 28% to 89% in Lp(A-I) and from 11% to 71% in Lp(A-I,A-II).

When plasma samples were adsorbed with anti-E immunoadsorbent, 99 ± 5% (n = 7) of plasma PLTP activity was detected in the apoE-free plasma (Table 1). Thus, minimal active PLTP was associated with apoE-containing lipoproteins in the seven plasma samples studied.

TABLE 1. PLTP activity in plasma depleted of apoA-I, apoA-II, or apoE

Sample	Percentage Plasma PLTP Activity		
	ApoA-I-Deficient Plasma	ApoA-II-Deficient Plasma	ApoE-Deficient Plasma
1	1.3	ND	ND
2	1.6	62.1	ND
3	2.7	51.1	ND
4	1.4	35.3	ND
5	3.5	85.2	98.4
6	0.6	89.4	104.6
7	1.2	83.3	104.7
8	0.5	28.6	89.5
9	ND	ND	98.9
10	ND	ND	97.2
11	ND	ND	99.2
Mean ± SD	1.6 ± 1.0	62.1 ± 24.8	98.9 ± 5.1

apoA-I, apolipoprotein A-I; ND, not done; PLTP, phospholipid transfer protein. Separate aliquots of the plasma samples were adsorbed with affinity-isolated anti-A-I, anti-A-II, or anti-E antibody to remove apoA-I-, apoA-II-, or apoE-containing lipoproteins, respectively. PLTP activity in plasma devoid of apoA-I, apoA-II, or apoE was determined and is expressed as percentage of original plasma activity.

To detect PLTP mass, apoA-I-free plasma, apoE-free plasma, and the lipoproteins bound to the anti-A-I and anti-E antibodies in two experiments were separated by SDS-gel electrophoresis, transferred to nitrocellulose membranes, and probed with a rabbit anti-human PLTP antibody, which has been shown to react strongly with active PLTP (14) and can inhibit >90% of plasma PLTP activity (20). **Figure 1** shows that when equal quantities of apoA-I-free plasma and apoE-free plasma were separated on the SDS gel, little PLTP was detected in the apoA-I-free plasma. In contrast, the PLTP mass in apoE-free plasma appeared to be comparable to that of the corresponding parent plasma samples. When equal plasma concentrations of apoA-I- and apoE-containing lipoproteins were separated on the SDS gel, considerably more PLTP was detected in the apoA-I-containing lipoproteins than in the apoE-containing lipoproteins. These observations are consistent with the activity results. Thus, anti-A-I immunoadsorbent removed nearly all PLTP activity as well as PLTP mass from plasma, whereas anti-E immunoadsorbent removed little plasma PLTP activity and PLTP mass.

PLTP activity in isolated HDL particles

The distribution of active PLTP between Lp(A-I) and Lp(A-I,A-II) isolated from DS-adsorbed apoB-free plasma was also studied. In 22 isolations, 3 ± 2% (range, 0.4–7%) and 25 ± 9% (range, 7–40%) of plasma PLTP activity were detected in Lp(A-I) and Lp(A-I,A-II), respectively, and 4 ± 4% (range, 0.4–17%) was detected in the flow-through lipoprotein-deficient plasma. To our surprise, 20 ± 9% (range, 8–34%) of plasma PLTP activity was also detected in the materials desorbed from the DS column. The minimal (3%) PLTP activity recovered in affinity-isolated Lp(A-I) and the unexpectedly high level of PLTP activity detected in the DS-bound materials were inconsistent with

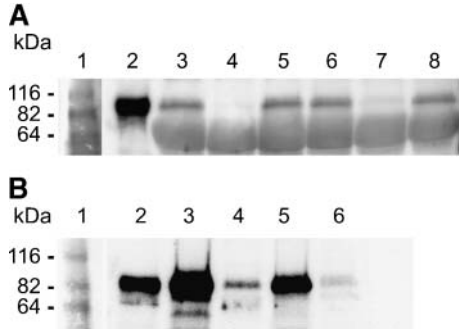

Fig. 1. Western blot analysis of plasma, apolipoprotein A-I (apoA-I)-free plasma, apoE-free plasma, apoA-I-containing lipoproteins, and apoE-containing lipoproteins. Plasma, apoA-I-free plasma, and apoE-free plasma, each containing 35 μg of total proteins, and 15μl of plasma equivalent of apoA-I- and apoE-containing lipoproteins were separated on a SDS 4–15% gradient gel under reducing conditions, transferred to a 0.2 μm nitrocellulose membrane, probed with a rabbit anti-phospholipid transfer protein (PLTP) antibody prepared in our laboratory, and detected with horseradish peroxidase-conjugated goat anti-rabbit IgG (Kirkegaard and Perry) and chemiluminescent substrates (Pierce). Samples in the lanes are as follows: (top panel) lane 1, Benchmark Prestained Protein Ladder (Invitrogen); lane 2, recombinant PLTP; lanes 3, 6, plasma from two individuals; lanes 4, 7, corresponding apoA-I-free plasma; lanes 5, 8, corresponding apoE-free plasma; (bottom panel) lane 1, Benchmark Prestained Protein Ladder; lane 2, recombinant PLTP; lanes 3, 5, apoA-I-containing lipoproteins of the two plasma samples in the top panel; lanes 4, 6, apoE-containing lipoproteins of the same two plasma samples.

the immunoadsorption data in Table 1 showing that >98% of plasma PLTP activity was associated with apoA-I-containing lipoproteins. These data are also inconsistent with previous reports demonstrating that nearly all PLTP activity in plasma is located in lipoproteins within the size range of HDL (12, 14), rather than in the size range of bulk LDL and VLDL. To investigate the size of the active PLTP removed by DS cellulose, the DS-bound fractions from two plasma samples were subjected to FPLC size-exclusion chromatography, and PLTP activity in the fractions was measured. **Figure 2** illustrates that although PLTP activity in these two plasma samples was detected primarily in HDL-sized particles, substantial PLTP activity in the materials eluted from DS was detected in fractions larger and smaller than HDL.

The FPLC data, along with the near absence of PLTP activity in Lp(A-I) isolated from DS-adsorbed plasma, suggested that DS removed PLTP from these particles to form new active PLTP-containing complexes larger and smaller than HDL. To test this hypothesis, HDL particles were isolated from 10 plasma samples without preadsorption with DS cellulose. With this protocol, 37 ± 11% (range, 15–49%), 17 ± 9% (range, 7–37%), and 3 ± 2% (range, 1–6%) of plasma PLTP activity were recovered in Lp(A-I), Lp(A-I,A-II), and the flow-through plasma that contained the VLDL and LDL, respectively. This relative distribution is consistent with the immunoadsorption data, which show that on average, more active PLTP is associated with Lp(A-I) than with Lp(A-I,A-II). Also, the minimal PLTP activity detected in the flow-through plasma containing the VLDL and LDL confirms that little active PLTP is associated with apoB-containing lipoproteins. When a preparation of Lp(A-I) and Lp(A-I,A-II) isolated from whole plasma was subsequently adsorbed with DS cellulose, only 40% and 58% of the starting PLTP activities in these particles could be detected in the post-DS-adsorbed Lp(A-I) and Lp(A-I,A-II), respectively. Upon size-exclusion chromatography, PLTP activity in Lp(A-I) and Lp(A-I,A-II) was detected in fractions corresponding to HDL, whereas the PLTP activity recovered from DS was detected in fractions larger and smaller than HDL (**Fig. 3**), confirming that DS can remove active PLTP from apoA-I-containing

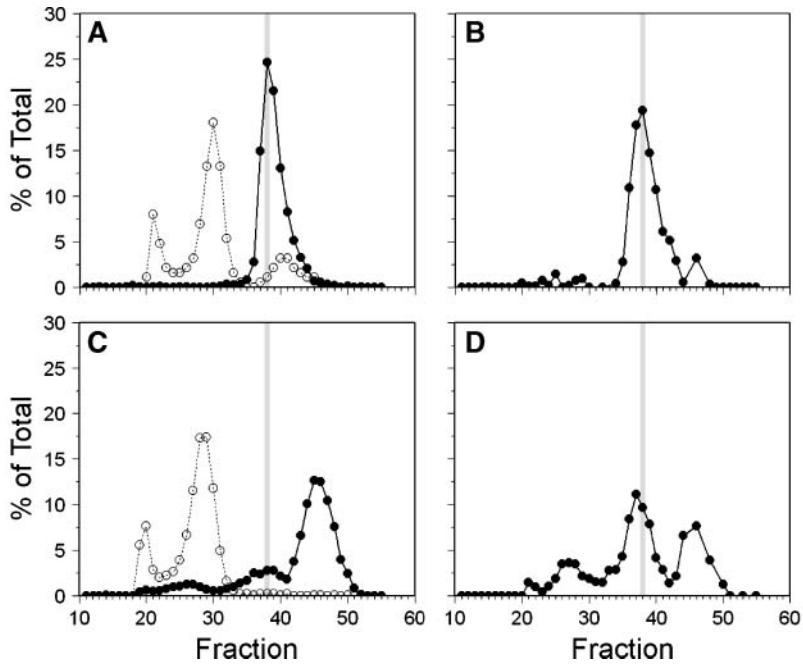

Fig. 2. Size profile of active PLTP removed from plasma by dextran sulfate (DS) cellulose. Plasma was adsorbed with DS cellulose to remove apoB-containing lipoproteins. Proteins bound to the DS were dissociated from the affinity medium, desalted, and concentrated. Two plasma samples (A, B) and the DS-bound fractions from the corresponding plasma samples (C, D) were separated by fast-protein liquid chromatography (FPLC). PLTP activity was measured in the fractions (closed circles). In one experiment, the cholesterol content of the fractions was also determined (open circles). PLTP activity and cholesterol in each fraction are expressed as percentage of total activity or cholesterol in all fractions. Gray bars identify the fractions with the highest PLTP activity when the plasma samples in (A) and (B) were separated by FPLC.

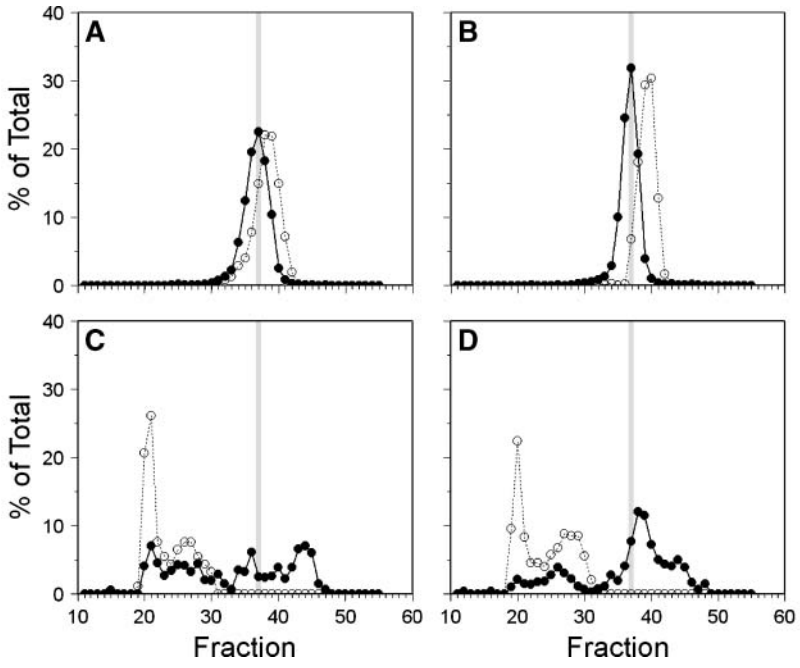

Fig. 3. Size profile of active PLTP removed from Lp(A-I) and Lp(A-I,A-II) by DS cellulose. Lp(A-I) and Lp(A-I,A-II) were isolated from whole plasma using anti-A-I and anti-A-II immunoadsorbents. These HDL particles were adsorbed with DS cellulose. Proteins bound to the DS cellulose were dissociated from the affinity medium, desalted, and concentrated. Lp(A-I) (A) and Lp(A-I,A-II) (B) after DS adsorption and the DS-bound materials from Lp(A-I) (C) and Lp(A-I,A-II) (D) were separated by FPLC. PLTP activity (closed circles) and cholesterol (open circles) in the fractions were measured and are expressed as percentage of total activity or cholesterol in all fractions. Gray bars identify the fractions with the most PLTP activity when Lp(A-I) (A) and Lp(A-I,A-II) (B) were separated by FPLC.

lipoproteins and that this process creates new active PLTP-containing complexes in vitro.

PLTP activity in DS-adsorbed plasma

Because the NaSCN used to desorb materials bound to anti-A-I, anti-A-II, and DS affinity columns appeared to partially inactivate PLTP, the extent of active PLTP removed by DS was also evaluated directly from DS-adsorbed plasma. In four experiments, when 5 ml of plasma was adsorbed with 10 ml of DS cellulose, only 17 ± 5% (range, 14–26%) of plasma PLTP activity was detected in the post-adsorbed plasma. Thus, DS removed 83 ± 5% of active PLTP from these four plasma samples.

DISCUSSION

Karkkainen et al. (13) were the first to report that macromolecular active PLTP complexes isolated from plasma samples contained apoE but not apoA-I, whereas macromolecular inactive PLTP complexes contained apoA-I but not apoE. Siggins et al. (22) also found that active PLTP isolated from HepG2-conditioned medium was associated with apoE but not apoA-I. They also reported the absence of inactive PLTP in HepG2-conditioned medium. These observations have led to the current working hypothesis that PLTP enters the circulation in an active form not associated with apoA-I but is associated with lipoprotein particles enriched with apoE. Active PLTP is converted into an inactive form in the circulation when it becomes associated with apoA-I-containing HDL-like particles during the transfer of surface remnants from triglyceride-rich lipoproteins to HDL (13, 15, 22). Our data, however, do not support this hypothesis. Our immunoadsorption studies using anti-A-I and anti-E antibodies (Table 1) clearly demonstrate that nearly all plasma PLTP activity is associated with apoA-I-containing lipoproteins and little plasma PLTP activity is associated with apoE-containing lipoproteins. Western blot analysis of apoA-I-free and apoE-free plasma further revealed that apoA-I immunoadsorbent also removed most of the PLTP mass from plasma, whereas the mass of PLTP in apoE-free plasma appeared to be comparable to that of the corresponding parent plasma samples. Also, the apoA-I-containing lipoproteins dissociated from the anti-A-I antibodies contained considerably more PLTP mass than the apoE-containing lipoproteins dissociated from the anti-E antibodies when equivalent plasma quantities of these lipoproteins were analyzed (Fig. 1). Considering that PLTP-mediated phospholipid transfer activity is operationally defined as the transfer of phosphatidylcholine from liposomes to bulk HDL in an in vitro assay system, it is reasonable to expect that active PLTP is associated with apoA-I-containing lipoproteins. Indeed, PLTP activity was present in the Lp(A-I) and Lp(A-I,A-II) isolated from whole plasma. The concept that apoA-I can inactivate active PLTP is not readily tenable with the PLTP activity assay, in which apoA-I-containing HDL particles are the acceptors in the system. It is also not consistent with in vivo evidence from a report showing that infusion of apoA-I/phospholipid discs in humans resulted in an increase of plasma PLTP activity despite an apparent decrease of PLTP mass (23).

What might have been the cause of the divergent findings between this and previous reports? We believe that a possible explanation lies in the following observations. *1*) DS affinity chromatography removed not only the apoB-containing lipoproteins but also as much as 83 ± 5% of plasma PLTP activity. *2*) Lp(A-I) isolated from whole plasma contained substantially more PLTP activity than Lp(A-I) isolated from DS-adsorbed plasma. *3*) DS removed 40–60%

of active PLTP from purified Lp(A-I) and Lp(A-I,A-II). *4*) The active PLTP removed by DS from whole plasma and from Lp(A-I) and Lp(A-I,A-II) formed new macromolecular complexes larger and smaller than bulk HDL. Similar to our use of DS cellulose, Karkkainen et al. (13) used heparin-Sepharose affinity chromatography as their first separation step to concentrate the PLTP in plasma. DS cellulose and heparin-Sepharose are known to bind apoB and apoE but not apoA-I (18, 24). Heparin-Sepharose is also the reagent used to separate HDL particles that contain apoE from those that do not (25). Heparan sulfate is analogous to DS with respect to its interaction with apoB- and apoE-containing lipoproteins. The negative *N*- and *O*-sulfo groups of the heparin participate in an ionic interaction with the positive charges of the amino acids lysine and arginine of the apolipoproteins (26). Similar to apoB and apoE, PLTP also contains heparin binding regions predicted from its primary amino acid sequence (27). Consistent with this prediction, we reported in an earlier study that heparin-Sepharose selectively binds PLTP but not CETP (28). Likewise, DS also does not bind CETP and LCAT (19), although it binds PLTP, as shown here. When plasma is subjected to heparin-Sepharose chromatography, a substantial amount of active PLTP would be removed along with apoE. Upon the release of these proteins from heparin-Sepharose, PLTP could associate with the apoE-containing particles in vitro to form newly created complexes that were not present in the original plasma. Therefore, we postulate that the association of active PLTP with apoE-containing macromolecular complexes, and the absence of apoA-I in these complexes reported by Karkkainen et al. (13), may be artifacts of their initial separation step. We do not have any data regarding the distribution of active PLTP in HepG2-conditioned medium. However, the use of heparin-Sepharose as the first step to concentrate PLTP in the conditioned medium (22) could have led to the similar conclusion that HepG2-derived active PLTP complexes are not associated with apoA-I but are enriched with apoE.

The distribution of PLTP activity between Lp(A-I) and Lp(A-I,A-II) has not been studied previously. Parallel anti-A-I and anti-A-II immunoadsorption experiments performed on seven plasma samples, and the measurement of PLTP activity in Lp(A-I) and Lp(A-I,A-II) isolated from whole plasma, showed that on average, more active PLTP is associated with Lp(A-I) than with Lp(A-I,A-II). This PLTP activity distribution qualitatively resembles the distribution of LCAT and CETP between these particles reported previously (21). However, the distribution of PLTP activity between these two HDL subpopulations varied considerably among the plasma samples studied. This large variation suggests that, as in the cases of LCAT (29) and CETP (30), the level and composition of Lp(A-I) and Lp(A-I,A-II) may affect the relative amount of active PLTP associated with these two populations of HDL particles.

Interaction of DS with LDL occurs primarily between the positive charges in apoB and the negative charges in DS (31). The DS cellulose used in this study is one of the common reagents used in LDL apheresis. Our data indicate that a substantial portion of plasma PLTP could be removed along with LDL in this process. This likely occurs via the interaction between the negative sulfo groups of DS and the positive amino acids in the heparin binding regions of PLTP. Adsorption of PLTP by DS can potentially affect lipoprotein metabolism and other PLTP-related metabolic processes in the post-LDL apheresis stage. The extent of this effect may depend on how efficiently the body is able to replenish the PLTP removed by this procedure.

In summary, we have shown that essentially all active PLTP in human plasma is associated with apoA-I-containing lipoproteins, distributed between particles with apoA-II and particles without apoA-II. The distribution of active PLTP between Lp(A-I) and Lp(A-I,A-II) varies widely among individuals. We have also shown that very little plasma active PLTP is associated with apoB-containing lipoproteins and apoE-containing lipoproteins. These observations do not support the current working hypothesis that the transfer of active PLTP from apoE-containing lipoproteins to apoA-I-containing lipoproteins results in the conversion of active PLTP to inactive PLTP. Finally, our observation that DS cellulose can remove active PLTP from plasma and from Lp(A-I) and Lp(A-I,A-II) to create new active PLTP complexes may provide an explanation for the divergent findings between this study and previous studies.

This study was supported by Grant HL-30086 from the National Institutes of Health. We thank the Northwest Lipid Research Laboratories for lipid and apolipoprotein measurements and Hal Kennedy for preparing the figures.

REFERENCES

1. Tall, A. R., S. Krumholz, and T. Olivercrona. 1985. Plasma phospholipid transfer protein enhances transfer and exchange of phospholipids between very low density lipoproteins and high density lipoproteins during lipolysis. *J. Lipid Res.* **26:** 842–851.
2. Tollefson, J. H., S. Ravnik, and J. J. Albers. 1988. Isolation and characterization of a phospholipid transfer protein (LTP-II) from human plasma. *J. Lipid Res.* **29:** 1593–1602.
3. Nishida, H. I., and T. Nishida. 1997. Phospholipid transfer protein mediates transfer of not only phosphatidylcholine but also cholesterol from phosphatidylcholine-cholesterol vesicles to high density lipoproteins. *J. Biol. Chem.* **272:** 6959–6964.
4. Tu, A. Y., H. I. Nishida, and T. Nishida. 1993. High density lipoprotein conversion mediated by human plasma phospholipid transfer protein. *J. Biol. Chem.* **268:** 23098–23105.
5. von Eckardstein, A., M. Jauhiainen, Y. Huang, J. Metso, C. Langer, P. Pussinen, S. Wu, C. Ehnholm, and G. Assmann. 1996. Phospholipid transfer protein mediated conversion of high density lipoproteins generates pre beta 1-HDL. *Biochim. Biophys. Acta.* **1301:** 255–262.
6. Wolfbauer, G., J. J. Albers, and J. F. Oram. 1999. Phospholipid transfer protein enhances removal of cellular cholesterol and phospholipids by high-density lipoprotein apolipoproteins. *Biochim. Biophys. Acta.* **1439:** 65–76.
7. Oram, J. F., G. Wolfbauer, A. M. Vaughan, C. Tang, and J. J. Albers. 2003. Phospholipid transfer protein interacts with and stabilizes ATP-binding cassette transporter AI and enhances cholesterol efflux from cells. *J. Biol. Chem.* **278:** 52379–52385.
8. Hailman, E., J. J. Albers, G. Wolfbauer, A. Y. Tu, and S. D. Wright. 1996. Neutralization and transfer of lipopolysaccharide by phospholipid transfer protein. *J. Biol. Chem.* **271:** 12172–12178.
9. Kostner, G. M., K. Oettl, M. Jauhiainen, C. Ehnholm, H. Esterbauer, and H. Dieplinger. 1995. Human plasma phospholipid

transfer protein accelerates exchange/transfer of α-tocopherol between lipoproteins and cells. *Biochem. J.* **205:** 659–667.
10. Desrumaux, C., V. Deckert, A. Athias, D. Masson, G. Lizard, V. Palleau, P. Gambert, and L. Lagrost. 1999. Plasma phospholipid transfer protein prevents vascular endothelium dysfunction by delivering alpha-tocopherol to endothelial cells. *FASEB J.* **13:** 883–892.
11. Damen, J., J. Regts, and G. Scherphof. 1982. Transfer of [^{14}C]-phosphatidylcholine between liposomes and human plasma high density lipoprotein. Partial purification of a transfer-stimulating plasma factor using a rapid transfer assay. *Biochim. Biophys. Acta.* **712:** 444–452.
12. Oka, T., T. Kujiraoka, M. Ito, T. Egashira, S. Takahashi, M. N. Nanjee, N. E. Miller, J. Metso, V. M. Olkkonen, C. Ehnholm, et al. 2000. Distribution of phospholipid transfer protein in human plasma: presence of two forms of phospholipid transfer protein, one catalytically active and the other inactive. *J. Lipid Res.* **41:** 1651–1657.
13. Karkkainen, M., T. Oka, V. M. Olkkonen, J. Metso, H. Hattori, M. Jauhiainen, and C. Ehnholm. 2002. Isolation and partial characterization of the inactive and active forms of human plasma phospholipid transfer protein (PLTP). *J. Biol. Chem.* **277:** 15413–15418.
14. Murdoch, S. J., G. Wolfbauer, H. Kennedy, S. M. Marcovina, M. C. Carr, and J. J. Albers. 2002. Differences in reactivity of antibodies to active versus inactive PLTP significantly impact PLTP measurement. *J. Lipid Res.* **43:** 281–289.
15. Janis, M. T., J. Metso, H. Lankinen, T. Strandin, V. M. Olkkonen, K-A. Rye, M. Jauhiainen, and C. Ehnholm. 2005. Apolipoprotein E activates the low-activity form of human phospholipid transfer protein. *Biochem. Biophys. Res. Commun.* **331:** 333–340.
16. Cheung, M. C., and J. J. Albers. 1984. Characterization of lipoprotein particles isolated by immunoaffinity chromatography: particles containing A-I and A-II and particles containing A-I but no A-II. *J. Biol. Chem.* **258:** 12201–12209.
17. Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA.* **76:** 4350–4354.
18. Yokoyama, S., R. Hayashi, M. Satani, and A. Yamamoto. 1985. Selective removal of low density lipoproteins by plasmapheresis in familial hypercholesterolemia. *Arteriosclerosis.* **5:** 613–622.
19. Cheung, M. C., and A. C. Wolf. 1989. In vitro transformation of apoA-I-containing lipoprotein subpopulations: role of lecithin: cholesterol acyltransferase and apoB-containing lipoproteins. *J. Lipid Res.* **30:** 499–509.
20. Cheung, M. C., G. Wolfbauer, and J. J. Albers. 1996. Plasma phospholipid mass transfer rate: relationship to plasma phospholipid and cholesteryl ester transfer activities and lipid parameters. *Biochim. Biophys. Acta.* **1303:** 103–110.
21. Cheung, M. C., A. C. Wolf, K. D. Lum, J. H. Tollefson, and J. J. Albers. 1986. Distribution and localization of lecithin:cholesterol acyltransferase and cholesteryl ester transfer activity in A-I-containing lipoproteins. *J. Lipid Res.* **27:** 1135–1144.
22. Siggins, S., M. Jauhiainen, V. M. Olkkonen, J. Tenhunen, and C. Ehnholm. 2003. PLTP secreted by HepG2 cells resembles the high-activity PLTP form in human plasma. *J. Lipid Res.* **44:** 1698–1704.
23. Kujiraoka, T., M. N. Nanjee, T. Oka, M. Ito, M. Nagano, C. J. Cooke, S. Takahashi, W. L. Olszewski, J. S. Wong, I. P. Stepanova, et al. 2003. Effects of intravenous apolipoprotein A-I/phosphatidylcholine discs on LCAT, PLTP, and CETP in plasma and peripheral lymph in humans. *Arterioscler. Thromb. Vasc. Biol.* **23:** 1653–1659.
24. Mahley, R. W., K. H. Weisgraber, and T. L. Innerarity. 1979. Interaction of plasma lipoproteins containing apolipoprotein B and E with heparin and cell surface receptors. *Biochim. Biophys. Acta.* **575:** 81–91.
25. Weisgraber, K. H., and R. W. Mahley. 1980. Subfractionation of human high density lipoproteins by heparin-Sepharose affinity chromatography. *J. Lipid Res.* **21:** 316–325.
26. Libeu, C. P., S. Lund-Karz, M. C. Phillips, S. Wehril, M. J. Hernaiz, I. Capila, R. J. Linhardt, R. I. Raffai, Y. M. Newhouse, F. Zhou, et al. 2001. New insights into the heparan sulfate proteoglycan-binding activity of apolipoprotein E. *J. Biol. Chem.* **276:** 39138–39144.
27. Day, J. R., J. J. Albers, C. E. Lofton-Day, T. L. Gilbert, A. F. Ching, F. J. Grant, P. J. O'Hara, S. M. Marcovina, and J. L. Adolphson. 1994. Complete cDNA encoding human phospholipid transfer protein from human endothelial cells. *J. Biol. Chem.* **269:** 9388–9391.
28. Albers, J. J., J. H. Tollefson, C. H. Chen, and A. Steinmetz. 1984. Isolation and characterization of human plasma lipid transfer proteins. *Arteriosclerosis.* **4:** 49–58.
29. Cheung, M. C. 1993. Distribution of lecithin-cholesterol acyltransferase in normolipidemic and dyslipidemic plasma. *Int. J. Clin. Lab. Res.* **23:** 30–33.
30. Moulin, P., M. C. Cheung, C. Bruce, S. Zhong, T. Cocke, H. Richardson, and A. R. Tall. 1994. Gender effects on the distribution of the cholesteryl ester transfer protein in apolipoprotein A-I-defined lipoprotein subpopulations. *J. Lipid Res.* **35:** 793–802.
31. Nishida, T., and U. Cogan. 1970. Nature of the interaction of dextran sulfate with low density lipoproteins of plasma. *J. Biol. Chem.* **245:** 4689–4697.