Distribution of phospholipid transfer protein in human plasma: presence of two forms of phospholipid transfer protein, one catalytically active and the other inactive

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Abstract **Plasma phospholipid transfer protein (PLTP) plays an important role in the maintenance of plasma high-density lipoprotein (HDL) content and remodeling of HDL in the circulation. In the present study we have used different fractionation methods to investigate the distribution of PLTP in human plasma. A novel enzyme-linked immunosorbent assay developed during the study allowed for simultaneous assessment of both PLTP mass and activity in the fractions obtained. Size-exclusion chromatography and plasma fractionation by nondenaturing polyacrylamide gel electrophoresis (PAGE) yielded similar results demonstrating that PLTP associates in native plasma with two distinct particle populations, while ultracentrifugation with high salt leads to detachment of PLTP from lipoprotein particles and loss of a majority of its phospholipid transfer activity. Interestingly, analysis of the size-exclusion chromatography fractions demonstrated that PLTP exists in the circulation as an active population that elutes in the position of HDL corresponding to an average molecular mass of 160 ± 40 kDa and an inactive form with an average mass of 520 ± 120 kDa. The inactive fraction containing approximately 70% of the total PLTP protein eluted between HDL and low density lipoprotein (LDL). Thus, the two PLTP pools are associated with different types of lipoprotein particles, suggesting that the PLTP activity in circulation is modulated by the plasma lipoprotein profile and lipid composition.—** Oka, T., T. Kujiraoka, M. Ito, T. Egashira, S. Takahashi, M. N. Nanjee, N. E. Miller, J. Metso, V. M. Olkkonen, C. Ehnholm, M. Jauhiainen, and H. Hattori. **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.* 2000. 41: 1651–1657.**

Supplementary key words plasma phospholipid transfer protein • plasma lipoprotein profile

A number of epidemiological studies have demonstrated that the plasma level of high density lipoprotein (HDL) is a predictor of coronary heart disease (CHD). Even though an inverse correlation between CHD risk and serum HDL levels has been well documented (1), the mechanism(s) underlying the antiatherogenecity of HDL are not clear. It has been proposed that the protective role of HDL is due to its role in reverse cholesterol transport, that is, transport of cholesterol from peripheral tissues to the liver (2). Although the mechanism of reverse cholesterol transport is far from resolved, several studies demonstrate that a subpopulation of HDL, preβ_1-HDL, is important in the uptake of cholesterol from tissues (3). Therefore, to reveal the mechanism(s) underlying the antiatherogenic potential of HDL it is essential to understand how individual HDL subpopulations are regulated and how they interrelate (4).

HDL are the smallest, most dense plasma lipoproteins. They originate from the liver and intestine (5). HDL are continuously remodeled during their circulation in plasma by factors that modulate their size, composition, and function (6). The remodeling also influences HDL metabolism and their cardioprotective properties. Proteins associated with some of the HDL subpopulations are

Abbreviations: apo, apolipoprotein; CETP, cholesteryl ester transfer protein; CHD, coronary heart disease; CHO, Chinese hamster ovary; ELISA, enzyme-linked immunosorbent assay; HDL, high density lipoprotein; HRP, horseradish peroxidase; kDa, kilodalton; LDL, low density lipoprotein; LPDP, lipoprotein-deficient plasma; MAb, monoclonal antibody; ND-PAGE, nondenaturing polyacrylamide gradient gel electrophoresis; OPD, *o*-phenylenediamine; PBS, phosphate-buffered saline; PC, phosphatidylcholine; PL, phospholipid; PLTP, phospholipid transfer protein; PVDF, polyvinylidene difluoride; rhPLTP, recombinant human PLTP; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TBS, Tris-buffered saline; VLDL, very low density lipoprotein.

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involved in the regulation of HDL subclass distribution. These include lecithin:cholesterol acyltransferase (7), phospholipid transfer protein (PLTP) (8), and cholesteryl ester transfer protein (CETP) (9). Together with hepatic lipase (10) and lipoprotein lipase (11) these proteins modulate the structure of HDL.

PLTP promotes the transfer of phospholipids between other lipoprotein classes and HDL (12). After hydrolysis of triglyceride-rich lipoproteins, surface remnant lipids are transferred to HDL and thus constitute precursors of HDL. This transfer is promoted by PLTP (13). The other function of PLTP in lipoprotein metabolism is to cause interconversion of HDL (14, 15). The interaction of PLTP with HDL results in the release of lipid-poor apolipoprotein A-I (apoA-I) particles with pre-β mobility and the formation of large HDL by a mechanism involving particle fusion (16). The small apoA-I particles formed are good acceptors of cell membrane cholesterol (17). The remodeling of HDL by PLTP is influenced by the protein composition of the particle (18) and is enhanced significantly by the presence of triglyceride in the HDL particles (19).

We have developed a sandwich enzyme-linked immunosorbent assay (ELISA) using two monoclonal PLTP antibodies to determine the concentration of PLTP mass in human plasma. Our studies revealed that in plasma from healthy subjects there is a discrepancy between PLTP mass and activity (20, 21). The present study was undertaken in order to elucidate mechanisms underlying this phenomenon.

In this study, we report that PLTP in human plasma exists in two forms, one active and the other inactive, which are associated with different macromolecular complexes.

MATERIALS AND METHODS

Materials

Egg phosphatidylcholine, bovine phosphatidylserine, and *o*-phenylenediamine (OPD) were from Sigma (St. Louis, MO). 1-Palmitoyl-2-[1-^{14}C]palmitoyl phosphatidylcholine (80–120 mCi/mmol) was from NEN Life Science Products (Boston, MA). Heparin (5000 units/ml) was from Mochida Pharmaceutical (Tokyo, Japan). Block Ace was from Snow Brand Milk Products (Tokyo, Japan). *N*-Hydroxysuccinimidobiotin (NHS–biotin) was from Pierce (Rockford, IL). Horseradish peroxidase (HRP)-conjugated rabbit anti-mouse IgG antibody was from Zymed Laboratories (San Francisco, CA). Monoclonal antibodies to apoE, apoA-I, and apoA-II were supplied from Nipponshoji (Ibaraki, Japan).

Isolation of lipoproteins

Lipoproteins [very low density lipoprotein (VLDL), $d < 1.006$ g/ml; low density lipoprotein (LDL), $1.006 < d < 1.063$ g/ml; HDL, $1.063 < d < 1.21$ g/ml; HDL_2, $1.063 < d < 1.125$ g/ml; HDL_3, $1.125 < d < 1.21$ g/ml] were isolated from human plasma by sequential ultracentrifugation in a Beckman (Fullerton, CA) Ti 50.2 rotor, using solid KBr to adjust the density (22). The washed HDL_3 was prepared by refloating at density $d = 1.21$ g/ml (45,000 rpm, 48 h). Each lipoprotein fraction and lipoprotein-deficient plasma (LPDP, $d > 1.21$ g/ml) were dialyzed against 10 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, pH 7.4 (TBS) and stored at 4°C.

Purification of recombinant human PLTP

Recombinant human PLTP (rhPLTP) was prepared in Chinese hamster ovary (CHO) cells and purified as described (23). Human plasma PLTP was purified as described (15).

Preparation of monoclonal antibodies against PLTP

Monoclonal antibodies (MAbs) to PLTP were prepared as follows. BALB/c mice were immunized with 25 μg of purified rhPLTP, and spleen cells of the mice were fused with Sp2/0 (24). The supernatants of hybridoma cells were screened by ELISA, using plates coated with purified rhPLTP (100 ng/well) and by immunoblotting. Positive hybridoma cells were cloned by limiting dilution and injected into Pristane-primed BALB/c mice. MAbs were purified from ascites with protein A-Sepharose CL-4B (Amersham Pharmacia Biotech, Uppsala, Sweden), and dialyzed against phosphate-buffered saline (PBS), and stored at −80°C. Specificity of MAbs 113 and 114 was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)/immunoblot analysis against purified human plasma PLTP and human plasma.

Fractionation of human plasma by size-exclusion chromatography

Human plasma from healthy volunteers was collected into EDTA tubes and immediately centrifuged for 20 min at 2,000 *g* at 4°C. The lipid profiles of subjects (n = 6) were as follows: total cholesterol, triglyceride, and HDL cholesterol were 4.13 ± 0.63 mM (mean ± SD), 1.08 ± 0.58 mM, and 1.35 ± 0.33 mM, respectively. PLTP concentration and activity of subjects (n = 6) were 14.8 ± 3.7 μg/ml, and 6.0 ± 0.5 μmol/ml/h, respectively. Plasma (1.5 ml) was applied to a fast protein liquid chromatography system consisting of two Superose 6HR 10/30 columns (Amersham Pharmacia Biotech) equilibrated with TBS, pH 7.4. Chromatography was performed at a flow rate of 0.25 ml/min and 0.5-ml fractions were collected.

SDS-PAGE, ND-PAGE, and immunoblotting

SDS-PAGE was performed on a 5–20% gradient polyacrylamide gel (ATTO, Tokyo, Japan). The samples were treated with 125 mM Tris-HCl (pH 6.8), 10% (w/v) glycerol, 2.3% (w/v) SDS, 0.01% bromophenol blue with 5% (v/v) 2-mercaptoethanol. Electrophoresis was performed in 25 mM Tris, 192 mM glycine, 0.1% SDS buffer at 20 mA for 1.5 h. The proteins were stained with Coomassie Brilliant Blue R-250 or electrotransferred to polyvinylidene difluoride (PVDF) with transfer buffer (25 mM Tris, 192 mM glycine, 20% methanol, 0.01% SDS) at 100 mA for 1 h and blocked with 5% (w/v) skim milk-PBS for 1 h.

Native PAGE was performed on a 5–20% gradient polyacrylamide gel (ATTO). The samples were treated with 40% (w/v) sucrose, 0.05% bromophenol blue. Electrophoresis was performed in TBE buffer (90 mM Tris-80 mM boric acid-3 mM EDTA, pH 8.3) at 150 V for 16 h at 4°C. To achieve effective transfer of proteins from the gel to the PVDF membrane, the gel was pretreated with 61.9 mM Tris, 20% (w/v) glycerol, 5% (v/v) 2-mercaptoethanol, 2.3% (w/v) SDS for 0.5 h, electrotransferred to PVDF, and blocked for 1h.

Immunoblotting was performed as follows: the PVDF membrane was incubated with monoclonal antibodies specific to PLTP. After washing the membranes were incubated with HRP-conjugated anti-mouse secondary antibodies (Zymed Laboratories). Bound antibodies were detected with an enhanced chemiluminescence kit (Amersham Pharmacia Biotech).

Measurement of phospholipid transfer activity

PLTP activity was measured as described (25). Liposomes were prepared as described (26). For the assay, 100 μl of diluted plasma (1:100) or 5 μl of the chromatography fractions was

added to 400 μl of reaction mixture containing HDL_3 (250 μg of protein) and PC liposomes (75 nmol of PC), and incubated at 37°C for 90 min. Liposomes were then precipitated with 300 μl of 230 mM NaCl, 92 mM $MnCl_2$, 150 U of heparin, and radioactivity in the supernatant was measured. Phospholipid transfer activity is expressed as the percentage of radiolabeled PC transferred to HDL_3. Assay was performed in duplicate and blanks without sample were subtracted. Duplicates of pooled human plasma, stored at −80°C, were also measured in each series as a reference. Throughout the study, the same batches of liposomes and HDL_3 were used. The PC transfer activity was linear up to 20% transfer of the total. The intra- and interassay (n = 8) coefficients of variation (CV) were less than 10%.

Measurement of PLTP mass

PLTP mass was measured by a sandwich ELISA using two monoclonal antibodies specific to PLTP. MAb 114 was used for capture and biotinylated MAb 113 was used for detection. The assay was performed as follows: 100 μl of monoclonal antibody 114 (5 μg/ml) in PBS was coated on plates (Immunoplate II MaxiSorp; Nunc, Roskilde, Denmark) by incubation at 4°C overnight. The wells were then blocked with 200 μl of PBS containing 4% Block Ace (Snow Brand Milk Products) for 2 h at room temperature. After washing the plate (PBS containing 0.1% Tween 20), 100 μl of standard solution or diluted plasma samples (1:200) or 5 μl of chromatography fractions was added and incubated for 2 h at room temperature. After washing, 100 μl of biotinylated monoclonal antibody 113 (1 μg/ml) was added to each well and incubated for 2 h at room temperature. After washing, 100 μl of HRP-conjugated streptavidin (1 μg/ml) (Vector Laboratories, Burlingame, CA) was added and incubated for 1 h, followed by incubation with 100 μl of substrate solution containing OPD (0.25 mg/ml) and 0.015% H_2O_2. After 30 min, the reaction was stopped by the addition of 100 μl of 2 N H_2SO_4. Absorbance at 492 nm was measured by a plate reader (Labsystems, Helsinki, Finland). A pooled culture medium from CHO cells expressing rhPLTP was used as a secondary standard and purified rhPLTP was used as a primary standard. The assay range of the ELISA method was from 0.6 to 15 ng of PLTP per well. The assay was performed in duplicate for each sample. The intra- and interassay (n = 8) coefficients of variation (CV) were less than 5%.

Analytical methods

The measurement of total cholesterol, triglyceride, and HDL-cholesterol in plasma was performed with an automated analyzer 7450 (Hitachi Instruments, Tokyo, Japan) and commercial kits (Daiichi Pure Chemical Industries, Tokyo, Japan).

Cholesterol content in the fractions obtained by size-exclusion chromatography was measured with a commercial kit (Wako Pure Chemical Industries, Osaka, Japan). Protein concentration was determined by bicinchoninic acid protein assay kit (Pierce, Rockford, IL) using bovine serum albumin (Bio-Rad) as a standard.

RESULTS

We have prepared two monoclonal antibodies (MAbs) specific to PLTP. SDS-PAGE and immunoblot analysis revealed that these antibodies recognized a single 80-kDa band in human plasma that corresponds to the mobility of purified human plasma PLTP (**Fig. 1**). The antibodies were used to develop a sandwich ELISA for PLTP mass determination. The assay enabled us to measure plasma PLTP mass in the range of 1.2 to 30 μg/ml.

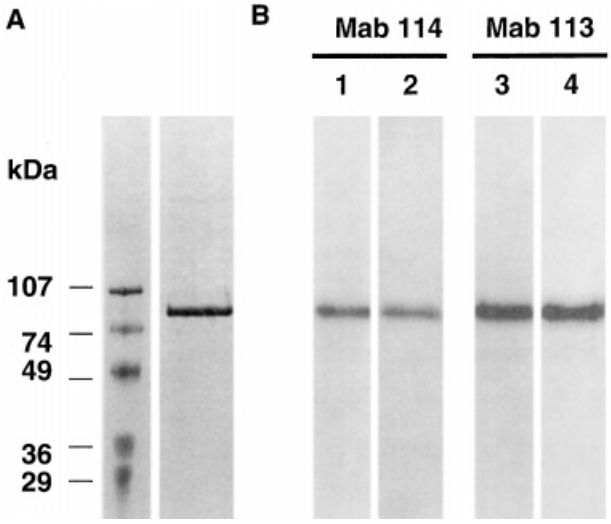

Fig. 1. Western blot analysis of human plasma and purified PLTP with anti-PLTP antibodies. (A) Purified human plasma PLTP (400 ng/lane) was subjected to SDS-PAGE in a 5–20% polyacrylamide gel under reducing conditions, and visualized by silver staining. Molecular weight markers are indicated on the left-hand side. (B) Purified human PLTP and human plasma were subjected to SDS-PAGE in a 5–20% gel under reducing conditions, followed by electrotransfer to PVDF membrane. Immunoblotting was performed with anti-PLTP monoclonal antibody 114 or 113. Lanes 1 and 3, purified human plasma PLTP (20 ng); lanes 2 and 4, human plasma (1 μl).

The distribution of phospholipid transfer protein (PLTP) in human plasma was analyzed by three methods, ultracentrifugation, nondenaturing polyacrylamide gradient gel electrophoresis (ND-PAGE), and size-exclusion chromatography. After ultracentrifugation of plasma, the main portion of PLTP activity (22.6% of total activity) was recovered in the lipoprotein-deficient plasma (LPDP) fraction, d > 1.21 g/ml. Only 0.12% of the PLTP activity was found in the HDL_3 fraction (n = 3). However, the main portion of PLTP mass (59.4% of total mass) was recovered in this fraction (**Table 1**). In the lipoprotein fractions, VLDL, LDL, and HDL_2, no PLTP activity or mass could be detected. When the HDL_3 fraction was subjected

TABLE 1. Distribution of PLTP activity and mass fractionated by ultracentrifugation

Fraction	PLTP			
	Activity	Recovery	Mass	Recovery
	μmol/h	%	*mg*	%
Plasma (400 ml)	2171	100	6.478	100
VLDL	ND	—	ND	—
LDL	ND	—	ND	—
HDL_2	ND	—	ND	—
HDL_3	2.7	0.12	3.854	59.4
HDL_3[a]	ND	—	1.439	22.2
LPDP (d > 1.21 g/ml)	490	22.6	0.781	12.1

PLTP activity and mass of the ultracentrifugally isolated fractions were calculated by ELISA and PC transfer assay. The results represent the means of triplicate determinations; ND, not detectable.

[a] Washed HDL_3.

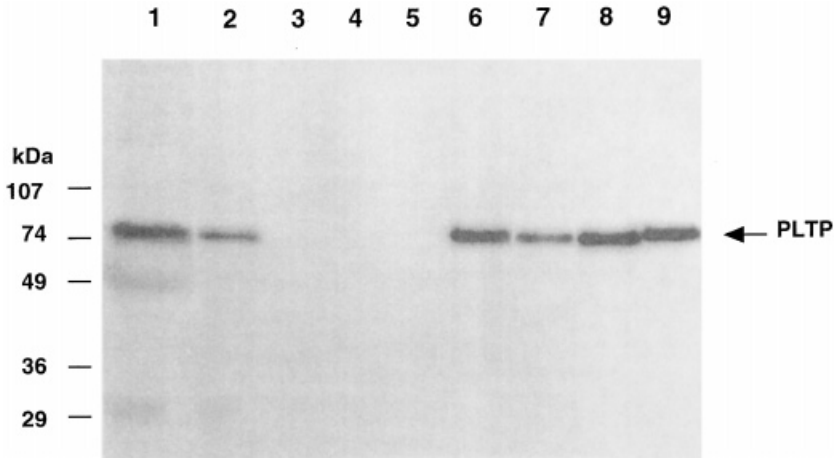

Fig. 2. Distribution of PLTP in the lipoprotein fractions isolated by ultracentrifugation. Human plasma and ultracentrifugally isolated lipoprotein fractions were subjected to SDS-PAGE in a 5–20% gel (ND-PAGE), followed by transfer to a PVDF membrane. PLTP protein was visualized with anti-PLTP monoclonal antibody 113. Lane 1, human plasma (1 μl); lane 2, LPDP (2 μl); lane 3, VLDL (5 μg); lane 4, LDL (5 μg); lane 5, HDL_2 (5 μg); lane 6, HDL_3 (5 μg); lane 7, washed HDL_3 (5 μg); lane 8, human plasma PLTP (20 ng); lane 9, rhPLTP (20 ng). Molecular weight markers are indicated on the left-hand side.

to a second cycle of ultracentrifugation at density 1.21 g/ml, no activity could be recovered in the HDL_3 fraction. However, PLTP protein was still detectable in washed HDL_3 (22.2% of total mass) by ELISA (Table 1) or immunoblotting, indicating a relatively strong interaction of PLTP with this HDL subclass (**Fig. 2**).

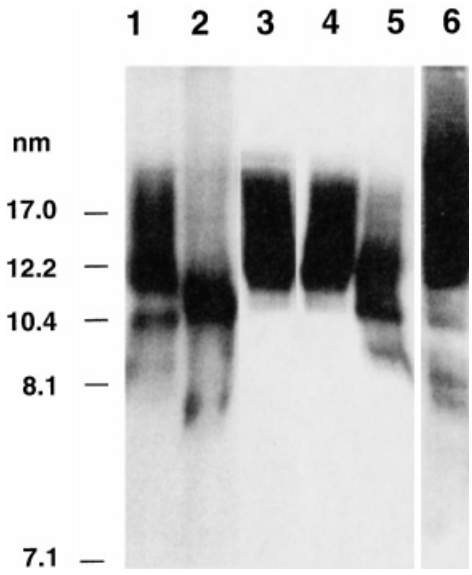

Fig. 3. Size distribution of particles containing PLTP as determined by nondenaturing PAGE. Human plasma and fractions obtained by ultracentrifugation or gel filtration were subjected to ND-PAGE in 5–20% gels. After electrophoresis, the proteins were transferred to a PVDF membrane and immunoblotted with monoclonal anti-PLTP antibody (MAb 113). Lane 1, human plasma (1 μl); lane 2, rhPLTP (20 ng); lane 3, HDL_3 (5 μg of total protein); lane 4, PLTP mass peak fraction (fraction 64) (10 μl); lane 5, PLTP activity peak fraction (fraction 74) (10 μl); lane 6, LPDP fraction (2 μl). Molecular weight markers are indicated on the left-hand side.

The distribution of PLTP protein in plasma and in the fractions isolated by ultracentrifugation was analyzed by a combination of ND-PAGE and immunoblotting with a specific anti-PLTP monoclonal antibody. After electrophoresis of human plasma, two populations of particles containing PLTP protein were observed, one in the size range 12.0 to 17.0 nm, while the size of the other ranged from 8.1 to 11.0 nm (**Fig. 3**). After ultracentrifugation, PLTP could be visualized in the HDL_3 fraction (12.0 to 17.0 nm). In the LPDP fraction obtained after centrifugation, two populations of particles containing PLTP protein were observed, one in the size range 12.0 to 17.0 nm, while the size of the other ranged from 8.0 to 11.0 nm (Fig. 3). rhPLTP purified from culture medium of transfected CHO cells could be visualized in the size range 10.7–12.0 nm.

To study the interaction of PLTP with lipoproteins under more physiological conditions, human plasma (n = 6) was fractionated by size-exclusion chromatography, and the eluted fractions were analyzed for PLTP activity and mass. A typical elution profile is depicted in **Fig. 4A**. The recovery of PLTP activity and mass in the size-exclusion chromatography was 84 ± 19 and 97 ± 14%, respectively. The activity eluted as one peak with a maximum at a position corresponding to an average molecular mass of 160 ± 40 kDa, ranging from 110 to 200 kDa. The elution profile of PLTP mass was quite different. The main portion of PLTP (almost 70%) was eluted between LDL and HDL with an average mass of 520 ± 120 kDa, ranging from 400 to 670 kDa. The fractions containing the major portion of PLTP protein (fractions 56–66; Fig. 4) displayed only marginal PLTP activity. The peak fractions containing maximal PLTP mass or activity were further analyzed by ND-PAGE and immunoblotting (Fig. 3). The PLTP mass peak (fraction 64) contained particles in the size range 12.0–17.0 nm, corresponding to the larger particles observed in human plasma. The PLTP activity peak (fraction 74) con-

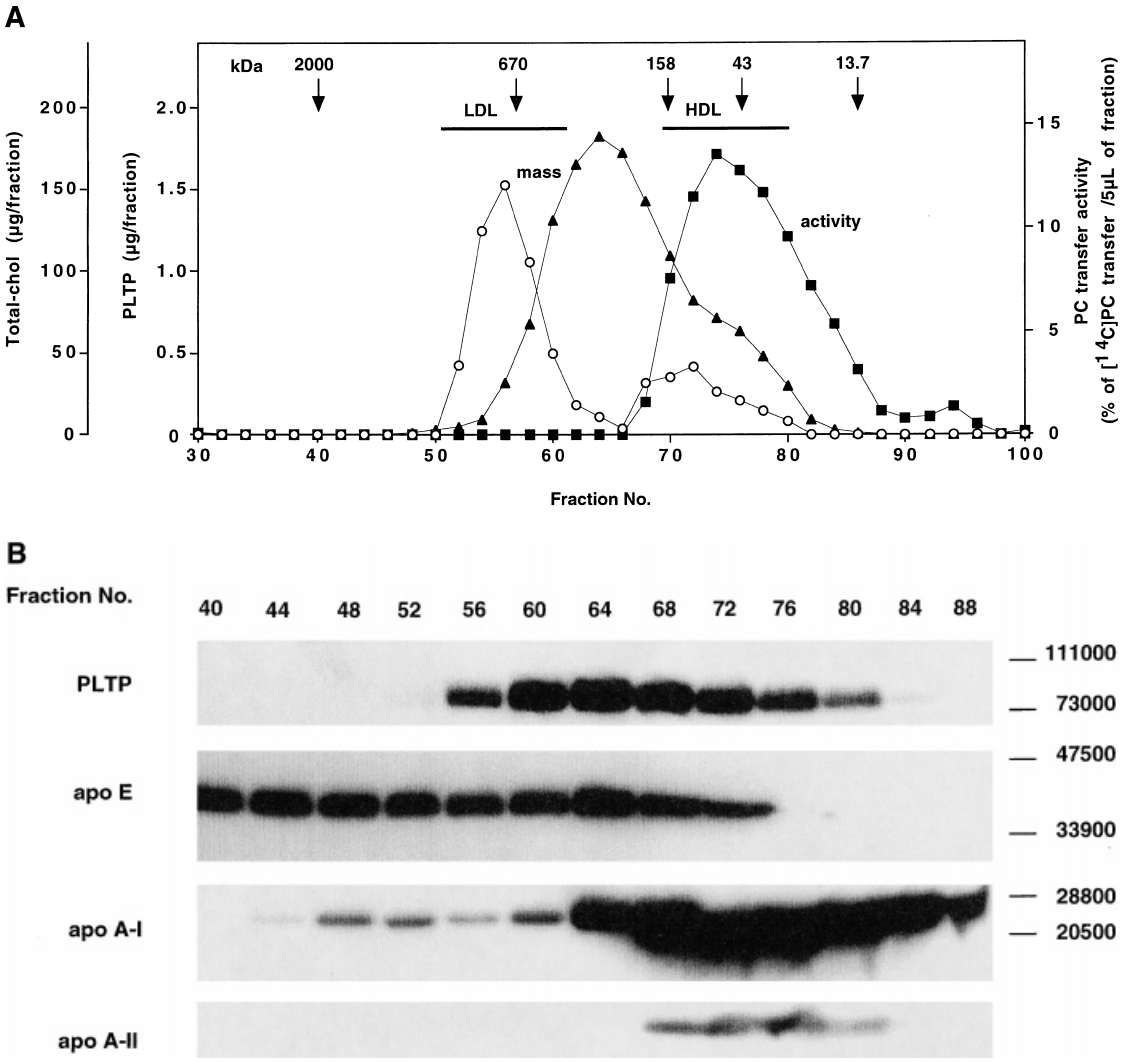

Fig. 4. Fractionation of human plasma by gel-filtration chromatography. (A) The eluted fractions (0.5 ml/fraction) were assayed for total cholesterol (open circles), PLTP activity (solid squares), and PLTP mass (solid triangles). (B) Immunoblot analysis of fractions by size-exclusion chromatography. To visualize PLTP, apoA-I, apoA-II, and apoE in the gel-filtration fractions, the fractions obtained by size-exclusion chromatography (10 μl/lane) were subjected to SDS-PAGE in 5–20% gel under reducing condition, and followed by electrotransfer to PVDF membrane. Immunoblotting was performed with anti-PLTP monoclonal antibody 113, anti-apoA-I antibody (MAb A-I-1), anti-apoA-II antibody (MAb A-II-9), and anti-apoE antibody (MAb E-6).

tained two particle populations, in the size range 9.7 to 15.4 nm, corresponding in size to the large and small particles detected in plasma.

As PLTP has been suggested to interact with the major HDL apolipoproteins, apoA-I and apoA-II, it was of interest to monitor the presence of these apoproteins in the eluted fractions (Fig. 4B). PLTP protein eluted in fractions 52–84, apoA-I in fractions 44–88, whereas apoA-II was located in fractions 68–84. ApoE was present in fractions 40–72, ranging from VLDL to large HDL.

DISCUSSION

We have observed that PLTP activity and mass in human plasma do not correlate (20, 21), suggesting that there may be varying amounts of a catalytically inactive form of PLTP in the circulation. This observation prompted us to investigate the distribution of PLTP activity and mass in plasma subfractions obtained by different methods.

Several studies have demonstrated that plasma PLTP activity is associated with a subfraction of HDL (27, 28) and may interact with the main HDL apolipoprotein, apoA-I (29). The present results and previous work (28) demonstrate that this interaction is largely interrupted by ultracentrifugation in the presence of high ionic strength. In addition, this procedure leads to a major loss of PLTP-mediated phospholipid transfer activity. To analyze the distribution of PLTP activity and mass in plasma under native conditions, plasma was fractionated by size-exclusion chromatography. Intriguingly, a major portion of PLTP protein (almost 70%) eluted in inactive form between the fractions containing LDL and HDL (molecular mass of 520 ± 120 kDa) whereas all the catalytically active PLTP

eluted as a separate peak with a molecular mass of 160 ± 40 kDa. This PLTP subpopulation eluted in a position corresponding to the size of HDL. In native plasma resolved on ND-PAGE a similar distribution of PLTP containing particles was observed, suggesting that the distribution in gel-filtration fractions reflects the physiological associations of PLTP.

Our results on the distribution of PLTP activity are in accordance with the previous findings of Speijer et al. (27). The novel observation here, the existence of a major inactive PLTP subpopulation, raises an interesting question concerning the molecular mechanisms responsible for PLTP activity modulation. The Western blotting analysis indicated that the PLTP protein in both the inactive and active peaks migrated identically at an approximated molecular mass of 80 kDa (Fig. 4B). This suggests that proteolytic cleavage of the protein (30) is not the cause of the inactive state. Furthermore, PLTP produced using a baculovirus/insect cell expression system and carrying carbohydrate chains highly different from those generated in mammalian cells is fully active (31), indicating that minor differences in glycosylation do not disturb the PC transfer activity. The results of ND-PAGE and immunoblotting suggest that the inactive and active forms of PLTP associate with the larger (>12.0 nm) and the smaller (<12.0 nm) particles, respectively (Fig. 3). It is plausible that differences in the activity of PLTP subpopulations are due to differential association with plasma lipoproteins, which may carry inactivating or activating lipid or protein components. Interestingly, it has been reported that the other plasma lipid transfer protein, CETP, can be inactivated by a lipid transfer inhibitory protein (apoF) present in LDL (32). It will be of interest to elucidate whether similar modulators can be identified for PLTP.

Studies in obese and diabetic subjects have indicated that increased PLTP activity appears to correlate with elevated levels of free fatty acids and triglycerides (33–35). Also, on alcohol use and cigarette smoking, increased PLTP activities have been observed with a concomitant elevation of plasma triacylglycerols (36, 37). Furthermore, our results demonstrate that the specific activity of PLTP (activity-to-mass ratio in circulation) correlates positively with plasma TG levels (20). Therefore, dyslipidemic states such as elevated triglycerides and/or cholesterol may modulate the distribution of active and inactive pools of PLTP. One can envision that, for example, on enhanced lipolysis leading to the formation of VLDL surface remnants enriched in phospholipids (38), PLTP may cotransfer with the PL-enriched remnants from the inactive pool to the HDL fraction, where it could be activated by an HDL-specific factor(s). Alternatively, PLTP may alter a turnover time more irreversibly to the inactive pool. In this case the plasma lipid status could modulate the turnover time of active PLTP.

Until now the distribution of PLTP in plasma has been based on the measurement of PL transfer activity only. In this way it has been impossible to gain insight into the regulation of PLTP function in the context of lipoprotein metabolism. One reason for this is obviously revealed by the present observation that plasma contains two forms of PLTP, differing in their compartmentalization and PL transfer activity. Elucidating the mechanisms involved in the generation of these two PLTP subpopulations is a challenging task for the future and will significantly contribute to our understanding of the regulatory networks controlling lipoprotein metabolism.

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