

Distribution of human plasma PLTP mass and activity in hypo- and hyperalphalipoproteinemia

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Abstract Plasma phospholipid transfer protein (PLTP) plays an important role in lipoprotein metabolism and reverse cholesterol transport. We have recently reported that plasma PLTP concentration correlates positively with plasma HDL cholesterol (HDL-C) but not with PLTP activity in healthy subjects. We have also shown that PLTP exists as active and inactive forms in healthy human plasma. In the present study, we measured plasma PLTP concentration and PLTP activity, and analyzed the distribution of PLTP in normolipidemic subjects (controls), cholesteryl ester transfer protein (CETP) deficiency, and hypo- α -lipoproteinemia (hypo-ALP). Plasma PLTP concentration was significantly lower (0.7 ± 0.4 mg/l, mean \pm SD, $n = 9$, $P < 0.001$) in the hypo-ALP subjects, and significantly higher (19.5 ± 4.3 mg/l, $n = 17$, $P < 0.001$) in CETP deficiency than in the controls (12.4 ± 2.3 mg/l, $n = 63$). In contrast, we observed no significant differences in plasma PLTP activity between controls, hypo-ALP subjects, and CETP deficiency (6.2 ± 1.3 , 6.1 ± 1.8 , and 6.8 ± 1.2 μ mol/ml/h, respectively). There was a positive correlation between PLTP concentration and plasma HDL-C ($r = 0.81$, $n = 89$, $P < 0.001$). By size exclusion chromatography analysis, we found that the larger PLTP containing particles without PLTP activity (inactive form of PLTP) were almost absent in the plasma of hypo-ALP subjects, and accumulated in the plasma of CETP deficiency compared with those of controls. These results indicate that the differences in plasma PLTP concentrations between hypo-ALP subjects, CETP deficiency, and controls are mainly due to the differences in the amount of the inactive form of PLTP.—Oka, T., S. Yamashita, T. Kujiraoka, M. Ito, M. Nagano, Y. Sagehashi, T. Egashira, M. N. Nanjee, K-i. Hirano, N. E. Miller, Y. Matsuzawa, and H. Hattori. **Distribution of human plasma PLTP mass and activity in hypo- and hyperalphalipoproteinemia.** *J. Lipid Res.* 2002. 43: 1236–1243.

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Epidemiological studies have demonstrated that the concentration of HDL cholesterol (HDL-C) is inversely related to the risk of coronary heart disease (CHD) (1, 2). HDL consists of heterogeneous particles, the size and composition of which are influenced by several factors, such as apolipoproteins, lecithin:cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein (CETP), phospholipid transfer protein (PLTP), hepatic triglyceride lipase, and lipoprotein receptors (3). It has been proposed that the protective actions of HDL against CHD reflect its role in reverse cholesterol transport (4). Several studies have demonstrated that a minor population of HDL, pre β ₁ HDL, is critical for the uptake of free cholesterol (FC) from tissues (5, 6). Esterification of FC by LCAT leads to maturation of HDL. The cholesteryl ester (CE) in HDL is transferred to apoB containing lipoproteins such as VLDL, IDL, and LDL by CETP, and LDL is catalyzed via hepatic LDL receptors. It has been shown that an HDL receptor, SR-BI, mediates the selective uptake of CE from HDL in the liver (7). PLTP is thought to be one of the important factors in lipoprotein metabolism in facilitating the transfer of phospholipids among lipoproteins, and in reverse cholesterol transport by generating pre β ₁-HDL (8). The role of PLTP in lipoprotein metabolism has been examined in PLTP transgenic and adenovirus-mediated PLTP overexpressed mice (9–14) and in PLTP knockout mice (15, 16).

Some genetic defects affect the plasma HDL-C concentration (17, 18). LCAT deficiency causes hypoalphalipoproteinemia (hypo-ALP) owing to impaired maturation of HDL (19). Tangier disease has recently been identified as

Abbreviations: ABCA1, ATP-binding cassette transporter A1; CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; FC, free cholesterol; HRP, horseradish peroxidase; hyper-ALP, hyperalphalipoproteinemia; hypo-ALP, hypoalphalipoproteinemia; PLTP, phospholipid transfer protein.

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a genetic defect in ATP-binding cassette transporter AI (ABCA1), which causes hypo-ALP due to diminished efflux of cholesterol from cells (20–24). Apolipoprotein A-I (apoA-I) deficiency causes hypo-ALP, because apoA-I is an integral component of HDL particles (25, 26). Familial HDL deficiency (FHD) is also documented, but the molecular basis of this is still unclear (27). Accelerated catabolism of HDL has been reported in some hypo-ALP subjects (28–33). In contrast, CETP deficiency causes hyperalphalipoproteinemia secondary to defective transfer of CE from HDL to apoB containing lipoproteins, resulting in accumulation of HDL-CE and enlargement of HDL particles (34). Delayed catabolism of HDL in CETP deficiency has also been reported (35).

Recently, we developed a sandwich ELISA using two monoclonal antibodies to measure human plasma PLTP concentration, and reported a positive correlation between plasma PLTP concentration and HDL-C in healthy Japanese subjects (36). In contrast, there was no correlation between plasma PLTP concentration and PLTP activity (36). A similar result was reported by Huuskonen et al. in Finnish subjects using a different sandwich ELISA (37). We have recently shown that PLTP exists in two forms; one is active and the other is inactive (38).

In the present study, we have measured plasma PLTP concentration and PLTP activity in normolipidemic subjects (as controls), hypo-ALP subjects, and CETP deficient subjects, and have analyzed the distributions of PLTP mass and PLTP activity in fractions of their plasma separated by size exclusion chromatography.

MATERIALS AND METHODS

Materials

Egg phosphatidylcholine, bovine phosphatidylserine, and *o*-phenylenediamine (OPD) human serum albumin were purchased from Sigma Chemical Company (St. Louis, MO). 1-Palmitoyl-2-[1-¹⁴C]palmitoyl phosphatidylcholine (DPPC, 80–120 mCi/mmol) was from NEN Life Science Products Inc. (Boston, MA). Heparin (5,000 U/ml) was from Mochida Pharmaceutical Co, LTD (To-

kyo, Japan). Horseradish peroxidase (HRP)-conjugated streptavidin was from Vector Laboratories, Inc. (San Francisco, CA). HRP-conjugated rabbit anti-mouse IgG antibody was from Zymed Laboratories, Inc. (San Francisco, CA). Prestained SDS-PAGE standards were from Bio-Rad Laboratories, Inc. (Hercules, CA). HMW electrophoresis calibration kit was from Amersham Pharmacia Biotech Inc. (Piscataway, NJ).

Subjects

Blood samples were obtained from subjects with hypoalphalipoproteinemia, including Tangier disease (TD) ($n = 2$), LCAT deficiency ($n = 2$), familial HDL deficiency (FHD) ($n = 3$), and apoA-I deficiency ($n = 1$), and from subjects with hyperalphalipoproteinemia owing to homozygous CETP deficiency ($n = 17$), in the Second Department of Internal Medicine, Osaka University, Japan. Two TD subjects, TD1 and TD2, and two FHD subjects, FHD2 and FHD3, shown in **Table 1** have been previously described for the ABCA1 gene mutation (39, 40). Plasma from another patient with familial LCAT deficiency ($n = 1$) was collected in London (41). Subjects with CETP deficiency were identified by screening plasma samples for CETP concentration by a sandwich ELISA (42), and the mutation of intron 14 G to A of the CETP gene was identified by PCR-reaction-restriction fragment polymorphism (42). CETP activity assays were carried out as previously described (42). Normolipidemic subjects ($n = 63$) were taken from our laboratory staff as controls. This study was approved by the ethical committee of Osaka University. Informed consent was obtained from all subjects. The plasma lipid profiles of subjects examined in this study are summarized in Table 1. Blood samples were taken after an overnight fast into EDTA-containing glass tubes (Terumo Corp., Tokyo, Japan), and were immediately centrifuged at 2,500 *g* at 4°C for 20 min. Plasma samples were stored at –80°C until used.

Isolation of lipoproteins

Lipoproteins were isolated from pooled fresh human plasma by sequential ultracentrifugation in a Beckman Ti 50.2 rotor using solid KBr to adjust the density (43). After ultracentrifugation, lipoproteins were dialyzed and stored at 4°C.

Measurement of PLTP mass and activity

PLTP concentration was measured by sandwich ELISA using two monoclonal antibodies specific for PLTP as previously described (36, 38). The assay range of the ELISA was from 0.6 to 15 ng/well. The sample was diluted adequately to adjust the measurable range. Assays were carried out in duplicate. The intra- and inter-assay coefficients of variation ($n = 5$) were less than

TABLE 1. Lipid profiles in hypo-ALP subjects, CETP deficiency, and controls

Subjects	Number (M / F)	Age (Years)	Total Cholesterol	HDL-C (mmol/l)	Triglyceride (mmol/l)	ApoA-I (g/l)	ApoA-II (g/l)	ApoB (g/l)
LCAT def. 1	M	30	3.83	0.02	4.09	0.36	0.09	0.81
LCAT def. 2	M	20	3.08	0.21	3.61	0.75	0.10	0.62
LCAT def. 3	M	57	3.08	0.34	2.46	0.42	0.05	0.31
TD 1	M	56	0.83	0.05	1.38	<0.10	0.03	0.42
TD 2	F	71	1.47	0.05	3.25	<0.10	0.01	0.15
ApoAI def. 1	F	30	3.03	0.18	0.41	<0.10	0.07	0.75
FHD 1	M	36	3.10	0.10	3.40	<0.10	0.02	1.26
FHD 2	F	53	2.77	0.18	1.67	0.22	0.13	0.97
FHD 3	M	48	2.48	0.13	1.94	<0.10	0.08	0.90
Hypo-ALP	9 (6/3)	45 ± 17	2.63 ± 0.92*	0.14 ± 0.10*	2.47 ± 1.21*	0.22 ± 0.24*	0.06 ± 0.04*	0.69 ± 0.35
CETP deficiency	17 (10/7)	45 ± 15	7.85 ± 0.89*	4.53 ± 0.83*	1.32 ± 0.56*	2.56 ± 0.41*	0.42 ± 0.13*	0.64 ± 0.13*
Controls	63 (34/29)	39 ± 13	4.45 ± 0.45	1.51 ± 0.30	0.95 ± 0.40	1.48 ± 0.20	0.32 ± 0.60	0.82 ± 0.12

M, Male; F, female. Data are represented as mean ± SD.

* $P < 0.001$; level of statistical significance by which data of hypo-ALP subjects or CETP deficiency differ from the data of controls (calculated by Student's *t*-test).

5%. In our ELISA for PLTP mass concentration, dose-dependency curves in samples of plasma (contains both inactive and active form of PLTP), washed HDL₃ (contains inactive form of PLTP alone), and recombinant human PLTP (contains active form of PLTP alone) were identical (Fig. 1), indicating that our sandwich ELISA react equally with the inactive and active forms of PLTP.

PLTP activity was measured by a liposome-HDL₃ system as previously described (36, 38). The assay range of PLTP activity was from 1.0 to 13.0 μmol/ml/h. Assays were carried out in duplicate. The intra- and inter-assay coefficients of variation (n = 5) were less than 10%.

Fractionation of human plasma by size-exclusion chromatography

Size exclusion chromatography was performed as previously described (38). Human plasma (1.5 ml) was fractionated by fast protein liquid chromatography, using two Superose 6HR 10/30 columns (Amersham Pharmacia Biotech, Uppsala, Sweden) connected in series and equilibrated with SMT buffer (10 mM Tris, 225 mM mannitol, 65 mM sucrose, 1 mM EDTA, pH 8.1) (44). Chromatography was performed at a flow rate of 0.25 ml/min, and 0.5 ml fractions were collected (38).

SDS-PAGE-Western blotting and non-denaturing-PAGE-Western blotting

SDS-PAGE and non-denaturing-PAGE were performed using a 5–20% gradient polyacrylamide gel (ATTO, Tokyo, Japan) as previously described (38). Western blotting was performed with anti-PLTP monoclonal antibody 113, followed by incubation with HRP conjugated rabbit anti-mouse IgG, as previously described (38). Bound antibodies were detected using a chemiluminescence reagent kit (NEN Life Science Products Inc., Boston, MA), and exposure on to X-ray film (Eastman Kodak Company, Rochester, NY).

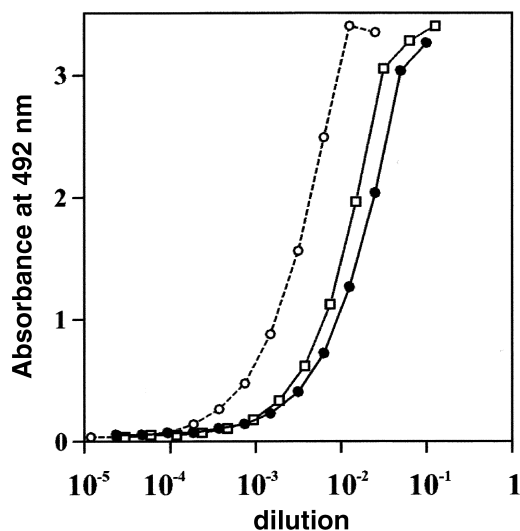


Fig. 1. Titration curve of the phospholipid transfer protein (PLTP) ELISA. The ELISA was performed as described in Materials and Methods. The titration curves were made using serial dilutions of 82.0 μg/ml of purified recombinant human PLTP (1:163840 to 1:40; open circle), 20.5 μg PLTP/ml of washed HDL₃ isolated by ultracentrifugation (1:8192 to 1:8; open square), or 15.4 μg/ml of human plasma (1:40960 to 1:10; closed circle). Each point represents the mean of duplicate determinations.

Other analytical methods

Total cholesterol, triglyceride, HDL-C, and apolipoproteins in plasma were measured by an automated analyzer Hitachi 7450 with commercial kits (Daiichi Pure Chemical Co. LTD., Tokyo, Japan).

Statistical analysis

The data are presented as mean ± SD. Inter-group differences in the parameters were analyzed by Student's *t*-test. Linear relationship was tested by Pearson's correlation coefficient. *P* < 0.001 were considered statistically significant.

RESULTS

PLTP mass and PLTP activity

Plasma PLTP concentration and PLTP activity in hypo-ALP subjects (n = 9), CETP deficiency (n = 17), and normolipidemic subjects (controls; n = 63) were measured. Plasma PLTP concentration was 12.4 ± 2.3 mg/l (mean ± SD), ranging from 7.4 to 17.2 mg/l in the controls. In hypo-ALP subjects, PLTP mass was very low (0.7 ± 0.4 mg/l, range 0.2–1.6 mg/l), whereas it was high in CETP deficiency (19.5 ± 4.3 mg/l, range 13.1–29.9 mg/l) (Table 2). In contrast, plasma PLTP activities were similar in these subjects: 6.2 ± 1.3 μmol/ml/h (range, 3.8–9.3 μmol/ml/h) in controls, 6.1 ± 1.8 μmol/ml/h (3.6–9.5 μmol/ml/h) in hypo-ALP subjects, and 6.8 ± 1.2 μmol/ml/h (4.7–9.6 μmol/ml/h) in CETP deficiency (Table 2).

Plasma PLTP concentration was positively correlated with the plasma concentrations of total cholesterol (*r* = 0.78, *P* < 0.001, n = 89), HDL-C (*r* = 0.81, *P* < 0.001), apoA-I (*r* = 0.91, *P* < 0.001), and apoA-II (*r* = 0.75, *P* < 0.001). In contrast, plasma PLTP activity did not correlate with any lipid parameter (data not shown). There was no correlation between plasma PLTP concentration and PLTP activity (*r* = 0.20, *P* = 0.11, n = 89). These results were consistent with those previously described in healthy subjects (36, 37).

TABLE 2. PLTP values in hypo-ALP subjects, CETP deficiency, and controls

Subjects	PLTP Mass (mg/l)	PLTP Activity (μmol/ml/h)
LCAT def. 1	0.2	5.1
LCAT def. 2	0.4	6.9
LCAT def. 3	0.5	5.8
TD 1	0.9	9.5
TD 2	0.5	7.1
ApoAI def. 1	1.0	4.3
FHD 1	0.7	7.0
FHD 2	1.6	5.3
FHD 3	0.8	3.6
Hypo-ALP	0.7 ± 0.4*	6.1 ± 1.8
CETP deficiency	19.5 ± 4.3*	6.8 ± 1.2
Controls	12.4 ± 2.3	6.2 ± 1.3

M, Male; F, female. Data are represented as mean ± SD.

**P* < 0.001; level of statistical significance by which data of hypo-ALP subjects or CETP deficiency differ from data of controls (calculated by Student's *t*-test).

SDS-PAGE-Western blotting and non-denaturing-PAGE-Western blotting

Typical results of SDS-PAGE-Western blot and non-denaturing-PAGE-Western blot analyses of plasma are depicted in **Fig. 2**. On SDS-PAGE-Western blot analysis, an identical size of PLTP protein (80 kDa) was detected in control, CETP deficiency, and hypo-ALP subjects (**Fig. 2A**, Lane 1–5). On non-denaturing-PAGE-Western blot analysis, two major populations of PLTP containing particles, small ones (particle size of 8.4 to 11.4 nm) and large ones (11.8 to 17.0 nm), were detected in controls (**Fig. 2B**, lane 1) as previously described (38). A similar distribution of PLTP containing particles was observed in CETP deficiency (8.1 to 11.0 nm and 11.8 to more than 17.0 nm), although increased size of larger PLTP containing particles compared with that of the controls was observed in a CETP deficient subject (**Fig. 2B**, lane 2). The distribution of PLTP containing particles in hypo-ALP subjects differed from that in a control and a CETP deficient subject. Smaller PLTP containing particles were predominant in hypo-ALP subjects (9.1 to 12.5 nm in an LCAT deficient subject, 9.1 to 11.0 and 12.2 nm in a TD subject, and 8.1 to 11.0 and 12.2 nm in a FHD subject) (**Fig. 2B**, lane 3–5).

Size exclusion chromatography

Typical elution profiles by size exclusion chromatography are depicted in **Fig. 3**. The recoveries of PLTP mass and

PLTP activity were $99 \pm 2\%$ and $81 \pm 22\%$ ($n = 2$) in control, $92 \pm 3\%$ and $70 \pm 7\%$ ($n = 2$) in CETP deficiency, $140 \pm 38\%$ and $72 \pm 13\%$ ($n = 3$) in hypo-ALP subjects, respectively. In controls and CETP deficiency, the peak of PLTP mass eluted between the size of LDL and HDL, while the peak of PLTP activity eluted in the size of HDL (**Fig. 3A**, **B**) as previously described (38). A slightly increased peak size and increased amount of the inactive form of PLTP compared with that in the controls was also observed in CETP deficiency (**Fig. 3B**). PLTP mass and its distribution in hypo-ALP subjects were significantly different to those in the controls and CETP deficiency. However, the amount of PLTP mass was quite low, and its distribution in the hypo-ALP subjects with LCAT deficiency, Tangier disease, and FHD showed that the peak of PLTP mass eluted in the size of HDLs (**Fig. 3C–E**). PLTP activity and its distribution in hypo-ALP subjects were rather similar to those in the controls and CETP deficiency (**Fig. 3A–E**).

We further examined the PLTP containing particle sizes in the fractions of size exclusion chromatography by non-denaturing PAGE-Western blotting (**Fig. 4**). In a control subject, larger PLTP containing particles (12.0 nm to more than 17.0 nm in size) were predominant in the fractions of 36–40 without PLTP activity (**Fig. 4A**, lane 2), and smaller PLTP containing particles (9.6 nm to 10.9 nm) appeared in the fractions of 41–70 (**Fig. 4A**, lane 3–8). Two PLTP containing particles, 10.8 and 12.6 nm in size, were

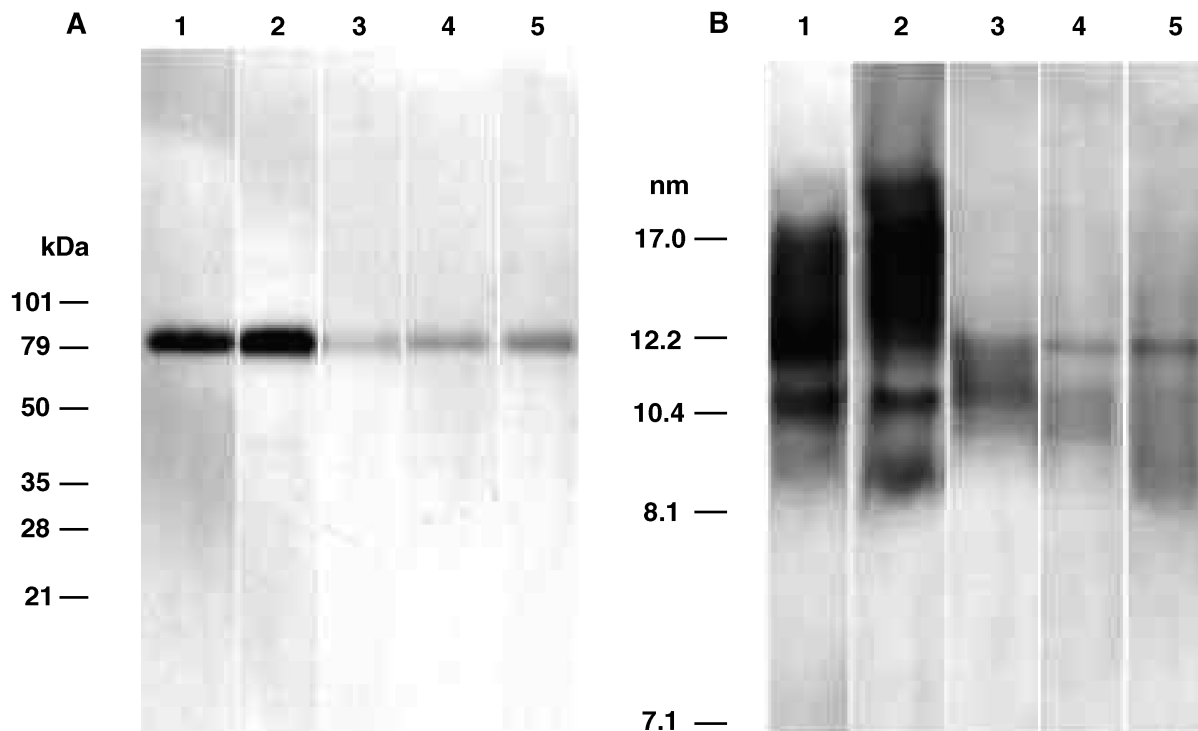


Fig. 2. Analysis of plasma PLTP protein and its particle size. Plasma PLTP protein was detected by SDS-PAGE-Western blotting (A) and the particle size of PLTP was analyzed by non-denaturing-PAGE-Western blotting (B). Plasma samples (1 μ l/lane) were subjected to SDS-PAGE under reducing condition or non-denaturing-PAGE in a 5–20% gel, followed by electrotransfer to a PVDF membrane, and Western blotting was performed using anti-PLTP monoclonal antibody 113 and horseradish peroxidase (HRP) conjugated anti-mouse IgG, as described in Materials and Methods. Lane 1, A normolipidemic subject; lane 2, a cholesteryl ester transfer protein (CETP) deficient subject; lane 3, an LCAT deficient subject; lane 4, a Tangier disease subject; lane 5, a Familial HDL deficient subject. Molecular markers or particle diameter markers are indicated on the left side of each figure.

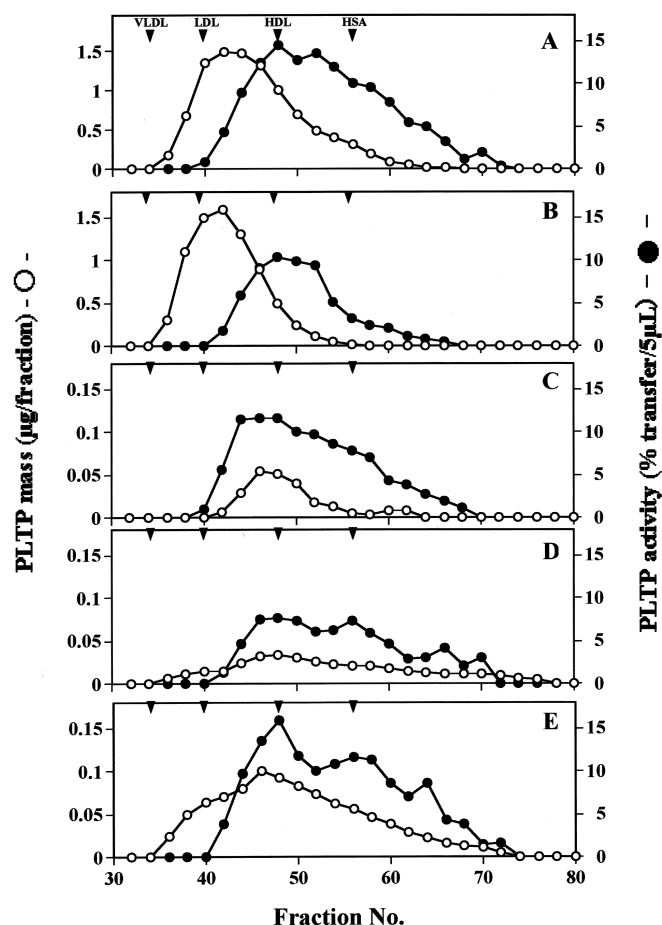


Fig. 3. The distribution of PLTP mass and activity in the size exclusion chromatography fractions. Plasma samples (1.5 ml) from a normolipidemic subject (A), a CETP deficient subject (B), an LCAT deficient subject (C), a Tangier disease subject (D), and a familial HDL deficient subject (E) were applied to two Superose 6HR columns connected in series, and were separated at a flow rate 0.25 ml/min; 0.5 ml of fractions were collected and analyzed. The subjects depicted in this figure are the same as those in Fig. 2. The distributions of PLTP mass (open circle) and PLTP activity (closed circle) were determined. PLTP mass concentration was measured with different sample volumes of the fractions; 5 μ l for controls and CETP deficiency; 50 μ l for hypoalphalipoproteinemia (hypo-ALP) subjects. PLTP activity was measured with 5 μ l of the fractions. Representative results for control ($n = 2$), CETP deficiency ($n = 2$), and hypo-ALP subjects ($n = 3$) were shown. Fast protein liquid chromatography analysis has performed twice for each sample. The elution positions of ultracentrifugally isolated VLDL, LDL, HDL, and human serum albumin are indicated by arrows at the top of each panel.

predominant in the fractions of 41–70 of hypo-ALP subjects (Fig. 4B–D). In addition, the fractions from LCAT deficient subject had smaller particles (9.0 nm in size).

DISCUSSION

This study has extended the previous observation that plasma PLTP concentration is positively correlated with HDL-C concentration, but not with PLTP activity in healthy subjects (36, 37). We have determined plasma PLTP concentration and PLTP activity, and have analyzed their distri-

bution in plasma by size exclusion chromatography in hypo-ALP subjects, CETP deficiency, and controls.

Plasma PLTP concentration was significantly lower in hypo-ALP subjects and significantly higher in CETP deficiency than in the controls. To our knowledge, these are the first reports on plasma PLTP concentration in hypo-ALP subjects and CETP deficiency. In contrast, there were no significant differences in plasma PLTP activity between the different groups of subjects. A similar result for plasma PLTP activity in Tangier disease has been reported by von Eckardstein et al. (45), but these are the first reports on plasma PLTP activity in LCAT deficiency, apoAI deficiency, FHD, and CETP deficiency.

Although plasma PLTP concentration was quite low in hypo-ALP subjects, we were able to detect the presence of PLTP and to confirm by SDS-PAGE and Western blotting that the molecular size of plasma PLTP protein in these subjects is identical to that in CETP deficiency and controls. Plasma PLTP activity in hypo-ALP subjects was completely inhibited by rabbit polyclonal antibody raised against purified recombinant human PLTP, as well as in CETP deficiency and controls (data not shown).

Non-denaturing-PAGE-Western blot analysis of plasma showed the presence of two size populations (large and small size) of PLTP containing particles in CETP deficiency and controls. Increased size of larger PLTP containing particles compared with that of the controls was observed in CETP deficiency, whereas the larger PLTP containing particles observed in controls and CETP deficiency were almost absent, and smaller PLTP containing particles predominated in hypo-ALP subjects.

Size exclusion chromatography showed the presence of the inactive form of PLTP in controls and CETP deficiency, as previously described (38). In CETP deficiency, an increased peak size and an accumulation of the inactive form of PLTP were observed. PLTP mass and its distribution in hypo-ALP subjects were significantly different from those in CETP deficiency and the controls. In hypo-ALP subjects, although the amount of PLTP mass was quite low, we could detect the presence of quite low amounts of the inactive form of PLTP in hypo-ALP subjects, and the peak of PLTP mass was almost in the same fraction as the peak of PLTP activity. In contrast to PLTP mass, PLTP activity and its distribution were rather similar in the hypo-ALP subjects, CETP deficiency, and controls. Non-denaturing-PAGE-Western blot analysis in plasma and size exclusion chromatography fractions also showed that larger PLTP containing particles (more than 12.0 nm in size) in the fractions without PLTP activity of control were predominant, which was consistent with the results reported by Murdoch et al. (46). There was an almost complete absence of larger PLTP containing particles without PLTP activity in those particles from hypo-ALP subjects. Smaller PLTP containing particles (less than 12.0 nm) were predominant in the fractions with PLTP activity of hypo-ALP subjects.

These observations suggest that the difference in plasma PLTP concentration between hypo-ALP subjects, CETP deficiency, and controls is mainly due to the differ-

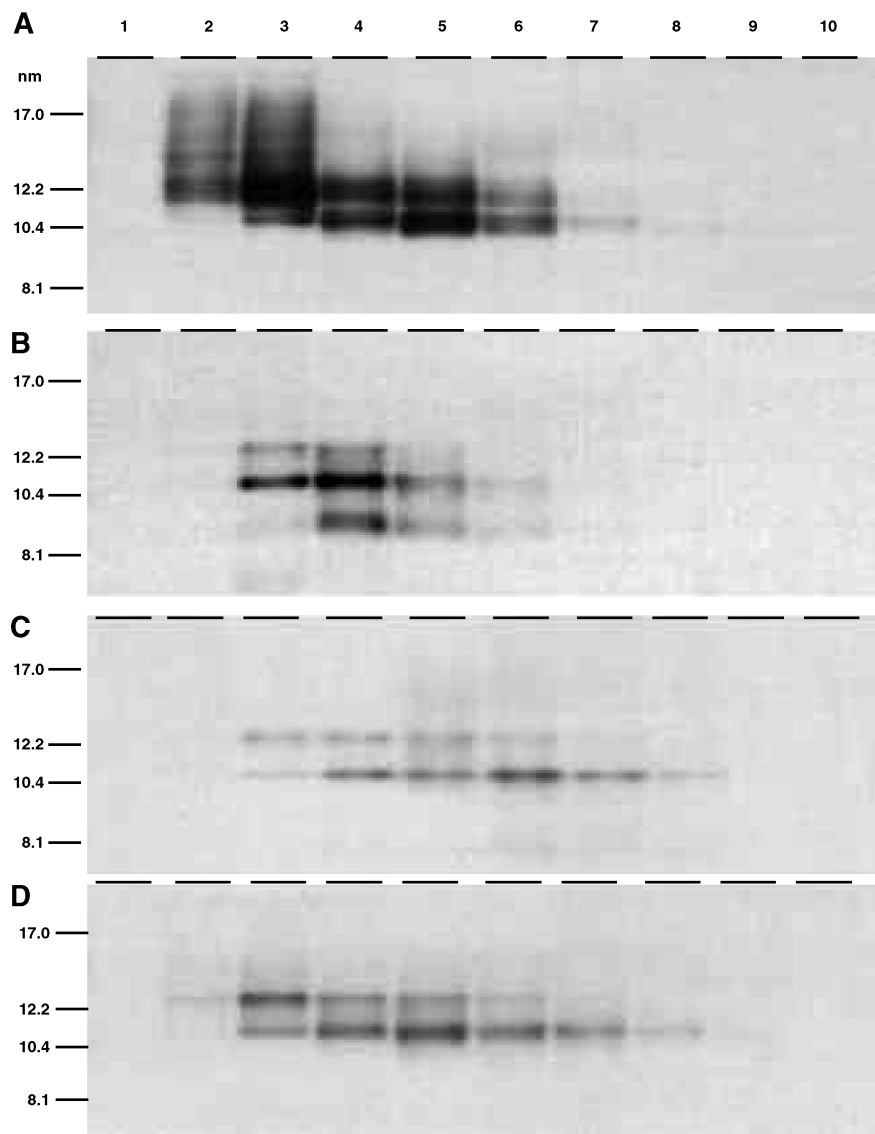



Fig. 4. Analysis of PLTP particle sizes in the fractions of size exclusion chromatography. The pooled fractions (10 μ l) by size exclusion chromatography of normolipidemic subject (A), an LCAT deficient subject (B), a Tangier disease subject (C), and a familial HDL deficient subject (D) were subjected (10 μ l/lane) to non-denaturing PAGE in a 5–20% gel. The subjects depicted in this figure are the same as those in Fig. 3. lane 1, fraction 31 to 35; lane 2, fraction 36 to 40; lane 3, fraction 41 to 45; lane 4, fraction 46 to 50; lane 5, fraction 51 to 55; lane 6, fraction 56 to 60; lane 7, fraction 61 to 65; lane 8, fraction 66 to 70; lane 9, fraction 71 to 75; lane 10, fraction 76 to 80. Molecular weight markers are indicated on the left side.

ence in the amount of the larger PLTP containing particles corresponding to the inactive form of PLTP.

In the previous paper, we reported that the inactive form of PLTP distributed in the HDL fraction isolated by ultracentrifuge (38). The partial characterization of inactive and active forms of PLTP isolated from healthy human plasma suggests that the inactive form of PLTP associates with apoA-I while active form of PLTP does not (47). These observations indicate that the inactive form of PLTP is on HDL particles; however, the particle size elution of the inactive form of PLTP is greater than the peak size of HDL by size exclusion chromatography. This could partly explain the positive correlation between plasma PLTP concentration and HDL-C across the spectrum of familial disorders that cause hypo-ALP and hyper-ALP. As HDL particles are known to increase in size during matu-

ration, the larger PLTP containing particles might be derived from the smaller ones during phospholipid transfer and/or fusion among lipoproteins.

In summary, we have found that plasma PLTP concentration is low in hypo-ALP subjects and high in CETP deficiency. In contrast, there were no differences in plasma PLTP activity among these subjects. The differences in plasma PLTP concentrations between hypo-ALP subjects, CETP deficiency, and controls analyzed in this study are mainly due to the differences in the concentrations of the larger PLTP containing particles (inactive form of PLTP). 

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