Binding of phospholipid transfer protein (PLTP) to apolipoproteins A-I and A-II: location of a PLTP binding domain in the amino terminal region of apoA-I

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Abstract The interaction of plasma phospholipid transfer protein (PLTP) with HDL has not been characterized in detail, although we have reported that the apoA-I/apoA-II molar ratio in the HDL particle influences PLTP-mediated HDL conversion, but not phospholipid transfer. The aim of this study was to examine whether PLTP binds apoA-I or apoA-II, and if this occurs, then determine the PLTP-binding domain of the apoA-I molecule. To study the PLTP/apolipoprotein interaction we used a solid phase ligand binding assay, the ELISA technique, and apoA-I and apoA-II affinity chromatography. PLTP bound to both apoA-I and apoA-II affinity columns, a finding subsequently utilized in the purification of PLTP. PLTP also bound to both apoA-I and apoA-II on ELISA plates in a concentration-dependent manner, and the binding could be displaced by preincubating the PLTP sample with purified apolipoproteins. To determine which portion of apoA-I is recognized by PLTP, we coated ELISA plates with either recombinant full-length apoA-I or three shortened apoA-I forms sequentially truncated from the C-terminus. To characterize the PLTP binding ability of the C-terminal region of apoA-I, we used both C-terminal CNBr-fragment and a synthetic C-terminal peptide of apoA-I. To further confirm the identity of the binding region, we probed the interaction with a polyclonal and several monoclonal anti-apoA-I antibodies. The antibodies that inhibited the interaction between PLTP and apoA-I were directed towards apoA-I epitopes localized between amino acids 27–141. The polyclonal antibody, R33, and the monoclonal antibody A-I-1 (epitope between amino acids 27–48) were most effective and reduced PLTP binding by 70%. These results show that PLTP binds to both apoA-I and apoA-II, and that the PLTP binding domain of apoA-I resides in the amino terminal region.—**Pussinen, P. J., M. Jauhiainen, J. Metso, L. E. Pyle, Y. L. Marcel, N. H. Fidge, and C. Ehnholm.** Binding of phospholipid transfer protein (PLTP) to apolipoproteins A-I and A-II: location of a PLTP binding domain in the amino terminal region of apoA-I. *J. Lipid Res.* 1998. **39:** 152–161.

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High density lipoproteins (HDL) are involved in the reverse transport of cholesterol from peripheral tissues to the liver, a pathway in which specific apolipoprotein (apo)A-I-containing lipoproteins, preβ_1Lp-A-I, have been shown to be the preferred acceptors of cellular cholesterol (1). There is in vitro evidence that plasma phospholipid transfer protein (PLTP) participates in this transport process by remodelling HDL particles and by regenerating primary cholesterol acceptors (2–4). This in vitro data is supported by studies in transgenic mice expressing human PLTP and apoA-I (5), in which the concentration of preβ-HDL particles in plasma was elevated dramatically. In addition to converting HDL into large and small particles, PLTP mediates net mass transfer of phospholipids (6) and α-tocopherol (7) between lipoproteins, and enhances cholesteryl ester transfer protein (CETP)-mediated transfer of cholesteryl ester from HDL to VLDL (8). The clinical significance of PLTP has recently been described, and marked changes were found in the lipoprotein profile accompanying PLTP deficiency (9). These observations suggest that PLTP may play a central role in lipoprotein metabolism and atherogenesis, and stimulate further studies to determine the physiological function of PLTP in lipoprotein metabolism.

Abbreviations: PLTP, phospholipid transfer protein; CETP, cholesteryl ester transfer protein; LCAT, lecithin:cholesterol acyltransferase; HL, hepatic lipase; apo, apolipoprotein; HDL, high density lipoprotein; MAb, monoclonal antibody; LpA-I, apolipoprotein A-I-containing lipoproteins; LpA-I/A-II, apoA-I and A-II-containing lipoproteins; preβ-HDL, preβ-migrating HDL; BSA, bovine serum albumin; HSA, human serum albumin; TBS, Tris-buffered saline; D, dalton; aa, amino acid.

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The mechanisms of PLTP-mediated phospholipid transfer remain uncertain, but some clues are provided by studies on the acyl chain and head group specificity of the phospholipids (10, 11). These studies suggest that PLTP does not form a tight complex with the phospholipid substrate. From preliminary observations, we obtained data supporting a mechanism that involves the release of apoA-I and some phospholipids from the surface of HDL leading to the fusion of apoA-I-depleted HDL particles (12). The presence of apoA-I appears essential for PLTP-mediated HDL conversion and fusion, as it was found that apoA-I-containing particles, but not particles containing only apoA-II were converted by PLTP (12, 13).

It has previously been reported that PLTP activity coelutes with a subfraction of HDL particles after gel filtration chromatography (14). However, the HDL counterparts that interact with PLTP are unknown at present. Because the apoA-I/apoA-II molar ratio in the HDL particle seems to have an important influence on the regulation of PLTP-mediated HDL conversion, the aim of the present study was to analyze the interaction of PLTP with these two major apolipoproteins of human HDL. In order to investigate this association, we used apoA-I and apoA-II affinity chromatography, ELISA plates coated with apoA-I and apoA-II, and a specific anti-PLTP antibody. To identify the PLTP-interacting domain of apoA-I, we have used *E. coli*-expressed apoA-I and its truncated forms, C-terminal CNBr-fragment or a synthetic C-terminal peptide of apoA-I, and probed the interaction with apoA-I-specific monoclonal antibodies.

MATERIALS AND METHODS

Reagents

1-Palmitoyl-2-[1-^{14}C]palmitoyl phosphatidylcholine (DPPC, specific activity, 55 mCi/mmol) was from Amersham, UK. Butyl-Toyopearl 650(M) was obtained from Merck, Germany, and RP-column C_{18} from Vydac, USA. The Superose 6HR gel filtration column, heparin-Sepharose CL-6B, CNBr-activated Sepharose 4B, ProteinG Sepharose 4B, Sephadex G-50 (superfine), and heparin-HiTrap columns were all obtained from Pharmacia, Uppsala, Sweden. The carboxyl terminal apoA-I peptide (5.3 kD, amino acids 198–243) was ordered from Chiron Mimotopes Pty. Ltd., Australia. CHAPS (3-[(3-cholamidopropyl)-dimethyl-ammonio]-1-propanesulfonate), egg phosphatidylcholine (PC), horseradish peroxidase, and BSA (ELISA-grade) were from Sigma. Heparin (5000 units/ml) was from Medica, Helsinki, Finland. All chemicals were of analytical grade.

Isolation of human apoA-I and apoA-II

Ultracentrifugally isolated HDL (d 1.063–1.21 g/ml) (15) was made 6 M with respect to guanidine hydrochloride. ApoA-I and apoA-II were purified with a minor modification of the method described by Scanu (16). The apoA-II fraction was delipidated overnight with ethanol–diethyl ether 3:1 (v/v) at −20°C in a volume ratio of 1:25 (17). The samples were subjected to ion-exchange chromatography on a DEAE-cellulose column (18). This protocol afforded lipid-free preparations of apoA-I and apoA-II according to analysis by SDS-PAGE (12.5%) stained with Coomassie Brilliant Blue. The purified apoproteins were dialyzed against 50 mM ammonium bicarbonate, pH 6.8, and lyophilized. The lyophilized proteins were dissolved in TBS and stored at −70°C.

CNBr-fragmentation of apoA-I

Cleavage and purification of the carboxyl terminal CNBr-fragment of apoA-I (10.7 kD, amino acids 148–243) were performed as described by Morrison, Fidge, and Grego (19). Briefly, 25 mg of pure apoA-I was digested with CNBr (0.5 g/ml) in 70% TFA. After incubation for 24 h under nitrogen in the dark at room temperature, the solution was dried under vacuum in a Speed-Vac, and dissolved in the column elution buffer (50 mM citrate, pH 3.8, 6 M urea). The sample was fractionated by Sephadex G-50 (superfine) column. The fractions containing the carboxyl terminal fragment of apoA-I (F4) were pooled and purified further by RP-HPLC (C_{18}). The peptide was eluted with a linear gradient (50 min) from 0 to 60% acetonitrile containing 0.1% TFA at a flow rate of 0.7 ml/min. Fractions were collected, dried, and analyzed by 15% SDS-PAGE.

Labeling of monoclonal antibody

ProteinG-Sepharose-purified monoclonal anti-pig-PLTP antibody G11 (IgG_1) was conjugated to horseradish peroxidase by the method of Avrameas and Ternynck (20). G11 (2 mg/ml) was dialyzed overnight against PBS. Ten milligrams of horseradish peroxidase was dissolved in 200 μl of PBS, containing 1.25% (v/v) glutaraldehyde, and incubated overnight at room temperature. The horseradish peroxidase solution was loaded onto a Superose 6HR column (30 cm × 1.5 cm) in PBS at a flow rate of 0.5 ml/min. The brown fractions were pooled and the volume of the pool was adjusted to 1 ml with PBS. The G11 (in 1 ml) was added to the peroxidase solution, followed by 100 μl of 1 M

carbonate-bicarbonate buffer, pH 9.5, and the mixture was incubated for 24 h at 4°C. The reaction was stopped with 100 μl of 0.2 M lysine and the mixture was incubated for a further 2 h at room temperature and dialyzed against PBS. The conjugated monoclonal antibody was precipitated by incubation with an equal volume of saturated ammonium sulfate, pH 7.0, for 6 h at 4°C. After centrifugation at 3,000 *g* for 30 min, the pellet was dissolved in 1 ml of PBS, and dialyzed against PBS. After dialysis, the sample was centrifuged at 10,000 *g* for 10 min, and the supernatant was adjusted to contain 1% BSA and 0.02% merthiolate. After filter-sterilization (0.45 μm filter) the conjugate was stored at 4°C.

PLTP binding to immobilized apoA-I and apoA-II

ELISA plates were coated with 5 μg/ml of plasma apoA-I, recombinant full-length and truncated apoA-I (−6–243, −6–222, −6–150, and −6–135) (21), apoA-I carboxyl terminal peptide (5.3 kD, amino acids 198–243), apoA-I carboxyl terminal CNBr-fragment (10.7 kD, amino acids 148–243), apoA-II, or HSA in carbonate buffer, pH 9.6, for 4 h, and post-coated overnight with 1% BSA in PBS at room temperature. The plate was washed six times with PBS. The PLTP samples obtained from the heparin-Sepharose (4,500 nmol/h/ml) were diluted in PBS, loaded into the wells with or without competitors, apoA-I, apoA-II, HSA, truncated apoA-I, apoA-I fragment, apoA-I peptide, or specific antibodies (see Table 1), and incubated for 2 h at room temperature. The plate was washed with PBS, containing 0.05% Tween 20 and finally, horseradish peroxidase-labeled G11 (22) was added in PBS, 1% BSA, 0.05% Tween 20. The plate was incubated for 2 h at room temperature and then overnight at 4°C, and washed six times with PBS, 0.05% Tween 20. Color development and assay for PLTP was completed by addition of horseradish peroxidase substrate.

PLTP isolation using apoA-I and apoA-II affinity chromatography

Purification was initiated from 1 l of pig plasma by precipitating plasma lipoproteins with dextran sulfate and $CaCl_2$ as described previously (3). Two early purification procedures, i.e., hydrophobic chromatography on a Butyl-Toyopearl 650(M) and affinity chromatography on a heparin-Sepharose column, have been described earlier (22). Active fractions from the heparin-Sepharose column were pooled, dialyzed against PBS (10 mM sodium phosphate buffer, pH 7.4, containing 150 mM NaCl), and after addition of protease inhibitors (1 mM benzamidine and 50 U/ml of aprotinin), the mixture was applied to an apoA-II–Sepharose column (25 mg of apoA-II coupled to 5 ml of CNBr-activated Sepharose CL-4B) by recycling of the sample overnight at a flow rate of 30 ml/h. The column was washed with PBS and the PLTP activity was eluted with 0.5% CHAPS in PBS at 10°C. The apoA-I–, and human serum albumin (HSA)–Sepharose columns were used under similar chromatographic conditions. The active fractions from the apoA-II column were combined, exhaustively dialyzed against 25 mM Tris-HCl, pH 7.4, containing 1 mM EDTA, and applied to a heparin-HiTrap column (1 ml), attached to a Merck HPLC-system, at a flow rate of 0.5 ml/min at room temperature. The column was washed with the dialysis buffer and the PLTP activity was eluted with an NaCl gradient (0–1 M) in the same buffer. Following this purification protocol, one major 78 kD band was visualized on a 12.5% SDS-PAGE. Inhibition of PLTP binding to apoA-I and apoA-II columns was studied by incubating a PLTP active pool from heparin-Sepharose chromatography with apoA-I or apoA-II for 1 h at room temperature before applying the mixture to the affinity column. In addition to desorption with 0.5% CHAPS, displacement of PLTP from apoA-I and apoA-II columns was studied by apoA-I, apoA-II, or HSA (0.4 mg/ml) as desorbents.

Other methods

Protein was determined by the method of Lowry et al. (23) with human serum albumin as standard. Human apoA-I and apoA-II (24) were assayed by immunoturbidometry, and CETP and PLTP activities were analyzed as previously described (25, 26). SDS-PAGE was performed by the Laemmli method (27), and Western blotting as described by Towbin, Staehelin, and Gordon (28).

RESULTS

Binding of PLTP to immobilized apoA-I and apoA-II

The interaction of PLTP with lipid-free apoA-I or apoA-II was studied by the ELISA method. Plates coated with either apoA-I, apoA-II, or HSA, which was used as a control for nonspecific binding, were incubated with serial dilutions of PLTP (0.3–0.7 mg/ml of total protein corresponding to PLTP activity of 800–4500 nmol/h/ml). PLTP/apolipoprotein binding was monitored with peroxidase-labeled monoclonal anti-pig-PLTP, G11. Binding of PLTP to both apoA-I and apoA-II was quantitatively similar and proportional to PLTP concentration (**Fig. 1A**). The plates coated with HSA (5 μg/ml) did not bind PLTP.

The specificity of the interaction was further analyzed by competition studies in which free apoA-I,

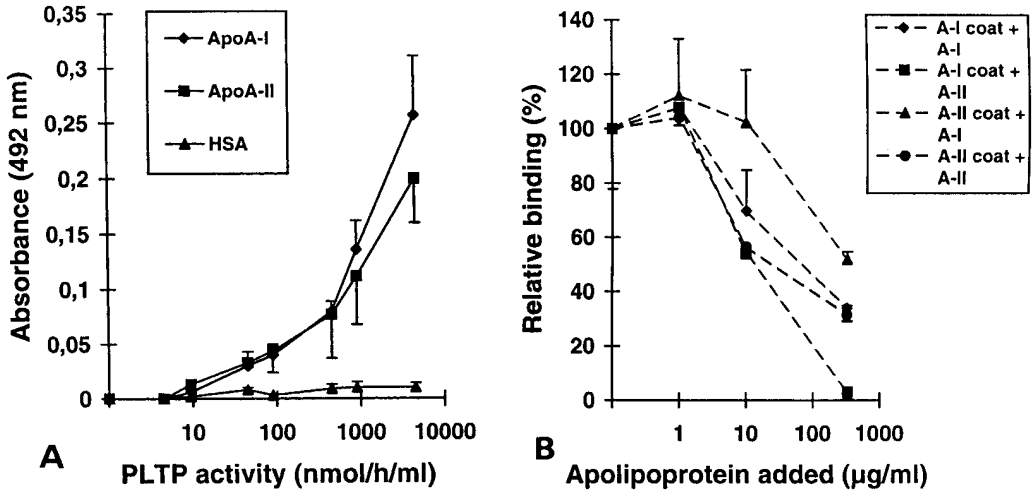

Fig. 1. A: Binding of PLTP to apoA-I and apoA-II immobilized on ELISA plates. ELISA plates were coated with 5 μg/ml of apoA-I, apoA-II, or HSA, and incubated with serial dilutions of PLTP (expressed as PLTP phospholipid transfer activity which was 4,500 nmol/h/ml in a non-diluted sample). The binding of PLTP was monitored with peroxidase-labeled monoclonal anti-pig PLTP, G11. B: The effect of apoA-I and apoA-II on PLTP binding. ELISA plates were coated with 5 μg/ml of apoA-I or apoA-II. Free apoA-I or apoA-II (1–330 μg/ml) was added to the PLTP sample (4,500 nmol/h/ml), which was incubated on the plate. The binding of PLTP was monitored with peroxidase-labeled anti-pig PLTP, G11. The mean values of three experiments in triplicate and the standard deviations are given.

apoA-II, or HSA (1–330 μg/ml) up to 66-fold excess over the amount used for coating the plates were added to the PLTP sample before addition to the wells (Fig. 1B). Addition of either apoA-I or apoA-II reduced PLTP binding to immobilized apoA-I in a dose-dependent manner, whereas HSA had no effect. ApoA-II at the highest amount used (330 μg/ml) totally blocked PLTP binding to apoA-I-coated plates. Also the binding of PLTP to plates containing immobilized apoA-II could be inhibited with apoA-I or apoA-II in a concentration-dependent manner.

Binding of PLTP to truncated forms of apoA-I

To investigate the binding domains of PLTP on apoA-I, ELISA plates were coated with recombinant full-length (residues −6–243) and three truncated forms of proapoA-I, terminating at residues 222, 150, and 135 (21). The concentration-dependent binding of PLTP was similar to full-length apoA-I and to the three truncated forms. As the truncations involved removal of varying proportions of the COOH-terminal half, it would appear that the NH_2-terminus of apoA-I is involved in the interaction with PLTP (**Fig. 2A**). This data was supported by competitive displacement experiments (Fig. 2B), which showed that the interaction of PLTP with apoA-I was reduced by addition of the truncated forms of apoA-I in a similar manner as that caused by the full-length apoA-I (Fig. 2B). At a concentration of 0.33 mg/ml, the truncated forms of apoA-I caused a 58–73% reduction in PLTP binding to immobilized full-length apoA-I.

Competitive ELISA with specific monoclonal apoA-I antibodies

Experiments with COOH-terminally truncated forms of apoA-I suggested that PLTP interacts with the amino terminal half of the apoA-I molecule. In order to test this hypothesis, we probed the interaction with both polyclonal and several monoclonal (MAb) anti-apoA-I antibodies (29–31) in a competitive type ELISA. The epitopes recognized by the MAbs are presented in **Table 1.** The apoA-I MAbs, which partially inhibited the interaction between PLTP and apoA-I, were all directed against residues between the 27–131 amino acid sequence of apoA-I (A-I-1, 2G11, 3G10, 3D4, and 5F6). The most effective competitors were MAb A-I-1 (amino acids 27–48) and polyclonal apoA-I antibody R33, which reduced PLTP binding to apoA-I by about 70% at a concentration of 0.1 μg/ml (**Fig. 3** and **Fig. 4**). Antibodies 3D4 and 3G10 share the same epitope on apoA-I (amino acids 98–121), but there is a marked difference in their ability to inhibit PLTP binding to apoA-I. This may be due to the difference between the area on apoA-I that the antibodies recognize: 3D4 covers the area continuously, but the epitope of 3G10 is discontinuous (31). The apoA-I MAbs, which did not inhibit the

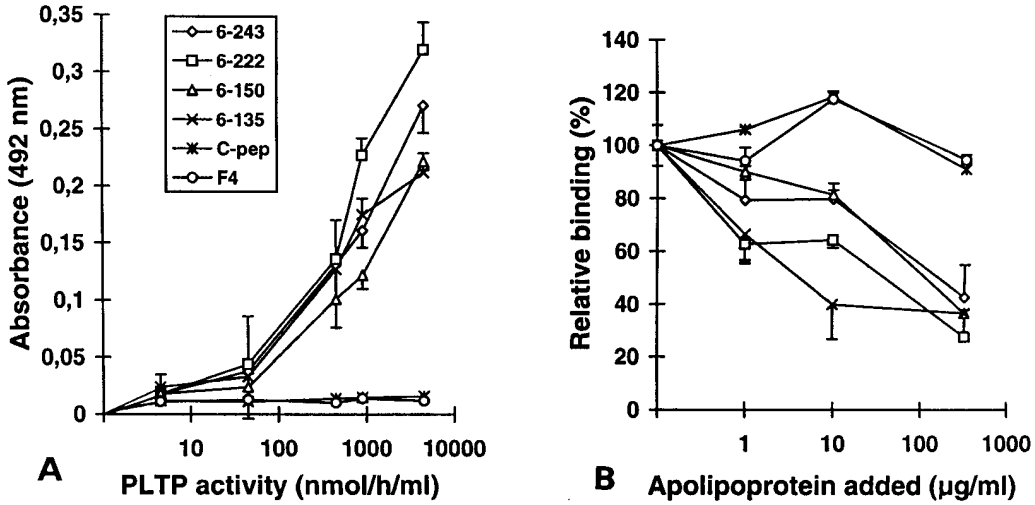

Fig. 2. A: Binding of PLTP to apoA-I fragments. ELISA plates were coated with 5 μg/ml of recombinant full-length pro-apoA-I (−6–243) and three truncated forms of it (−6–222, −6–150, −6–135), carboxyl terminal CNBr-fragment of apoA-I (10.7 kD, amino acids 148–243) (F4), or apoA-I peptide (5.3 kD, amino acids 198–243) (C-pep). Serial dilutions of PLTP were incubated on the plate and the binding of PLTP was monitored as in Fig. 1. B: The effect of apoA-I fragments on PLTP binding to immobilized apoA-I. ELISA plates were coated with 5 μg/ml of purified apoA-I. Full-length pro-apoA-I (−6–243), its three truncated forms (−6–222, −6–150, −6–135), carboxyl terminal CNBr-fragment (F4), or apoA-I peptide (C-pep) (1–330 μg/ml) were added to the PLTP sample (4,500 nmol/h/ml), which was incubated on the plate. The binding of PLTP was monitored. Mean values of two experiments in triplicate and the standard deviation are given.

PLTP/apoA-I interaction, were directed towards the antigenic epitopes localized on the carboxyl terminal half of apoA-I between amino acids 137–220 (M9, 4A12, and A-I-4) or the most proximal amino terminus residues (amino acids 2–8, 4H1) (Fig. 3). These results were consistent with the assumption that the PLTP binding site is localized in the amino terminal half of apoA-I (Fig. 4).

Binding of PLTP to the carboxyl terminal region of apoA-I

To further probe the identity of PLTP-binding domains of apoA-I, we digested apoA-I with CNBr and isolated the carboxyl terminal fragment with a molecular mass of 10.7 kD (amino acids 148–243). We used this fragment, along with a synthetic carboxyl terminal peptide of apoA-I (5.3 kD, amino acids 198–243), in binding and displacement experiments using ELISA. The results were consistent with our observation that the amino terminal portion of apoA-I is responsible for the PLTP binding. The C-terminal fragment or the synthetic peptide neither bound PLTP (Fig. 2A) nor inhibited its binding to apoA-I (Fig. 2B).

Use of apoA-I and apoA-II affinity chromatography in PLTP isolation

To determine whether binding of PLTP to either/or apoA-I and apoA-II could be exploited for purification purposes, we prepared affinity columns by coupling purified apoA-I and apoA-II to Sepharose CL-4B column as described in Methods. PLTP was partially purified from pig plasma, which first involved precipitating plasma with dextran sulfate/$CaCl_2$, followed by Butyl-Toyopearl 650M and heparin-Sepharose chromatographies as described before (22). PLTP-active fractions from heparin-Sepharose column were applied to apoA-I or apoA-II columns. A large proportion of the applied PLTP activity (66%) bound to the apoA-I column, and could be recovered (84%) by elution with 0.5% CHAPS in PBS. Of the activity not retained (44%) by the first passage through the apoA-I affinity column, a further 70% was bound to a re-generated apoA-I column. Columns containing bound apoA-II produced similar re-

TABLE 1. Epitopes of the monoclonal apoA-I antibodies

Name	Epitope (Amino Acids)	Reference
A-I 1.	27–48	29
A-I 4.1	211–220	29
M9	137–144	30
2G11	25–96	31
3D4	98–121	31
3G10	98–121	31
4A12	173–205	31
4H1	2–8	31
5F6	118–141	31

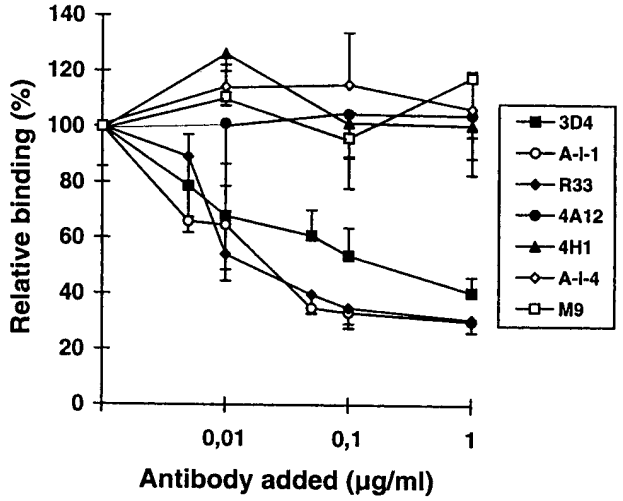

Fig. 3. The effect of apoA-I-specific monoclonal antibodies on binding of PLTP to immobilized apoA-I. ELISA plates were coated with 5 μg/ml of apoA-I, PLTP samples (4,500 nmol/h/ml) with added anti-apoA-I antibody (0.005–1 μg/ml) were incubated on the plate, and the binding of PLTP was monitored. The antibodies used are 3D4, A-I-1, R33, 4A12, 4H1, A-I-4, and M9, and their specific epitopes are summarized in Table 1. Mean values of two experiments in triplicate and the standard deviations are given.

sults; of the PLTP activity applied, 83% was bound to the apoA-II column. As for pig PLTP, human PLTP binds to both apoA-I and apoA-II immobilized in a column (data not shown).

Because fewer contaminating proteins coeluted with PLTP when apoA-II was the ligand, the apoA-II affinity chromatography step was subsequently chosen for isolating PLTP. The bound fraction contained PLTP as a major band with 2–3 minor contaminating protein bands (**Fig. 5,** lane B). This chromatography step resulted in a further 30-fold purification over that obtained by hydrophobic interaction and heparin-Sepharose chromatography (22). After apoA-II affinity chromatography the preparation could be further enriched by heparin-Sepharose (heparin-HiTrap) affinity chromatography, which resulted in one major 78 kD band visualized on a 12.5% SDS-PAGE (Fig. 5, lane C).

DISCUSSION

Association of proteins involved in cholesterol metabolism with specific plasma lipoprotein populations may provide clues to their physiological functions. Good examples are the HDL-associated proteins paraoxonase, apoJ, LCAT, CETP, and PLTP, which have all been assigned physiological roles in HDL metabolism. HDL comprises two main populations that differ in their apolipoprotein composition: one contains only apoA-I (LpA-I), while the other contains both apoA-I and apoA-II (LpA-I/A-II) (32). It has been suggested

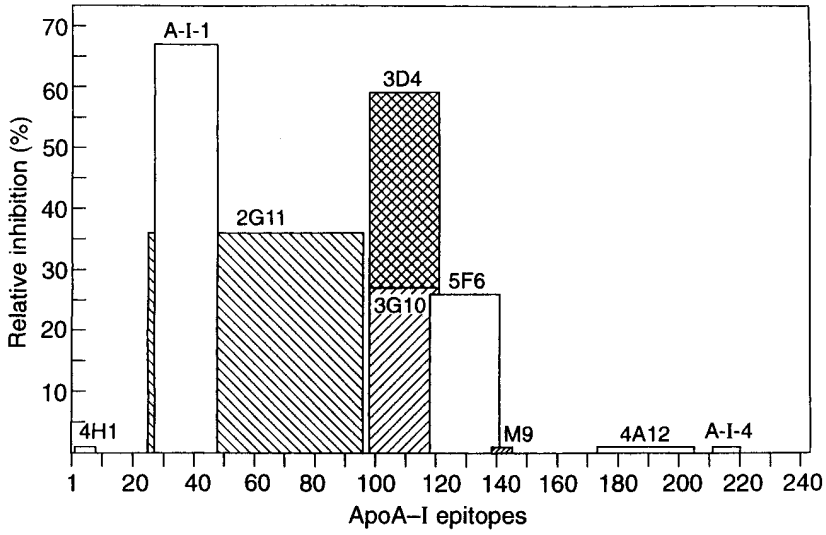

Fig. 4. Determination of the PLTP-binding domain in apoA-I using monoclonal anti-apoA-I antibodies. ELISA plates were coated with 5 μg/ml of apoA-I, PLTP (4,500 nmol/h/ml) samples with anti-apoA-I antibody (1 μg/ml) were incubated on the plates, and the binding of PLTP was measured using peroxidase-labeled monoclonal anti-PLTP, G11. Horizontal axis represents the amino acid chain of apoA-I. Width of the boxes shows the epitope on apoA-I, and height of the boxes represents the relative inhibition of PLTP binding to apoA-I by the corresponding antibody.

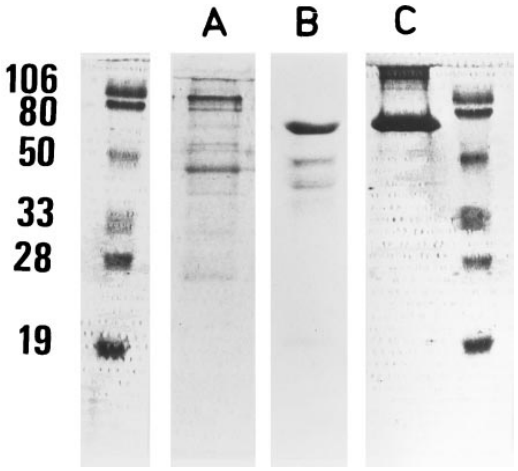

Fig. 5. 12.5% SDS-PAGE of purified pig plasma PLTP. Lane A: Active fractions from apoA-I affinity column. Lane B: Active fractions from apoA-II affinity column. Lane C: PLTP after apoA-II and heparin HiTrap chromatography. Molecular weight standards are on both sides of the gel.

that human plasma LpA-I/A-II and LpA-I have different physiological properties with regard to the ability of these particles to participate in reverse cholesterol transport and consequently the role they play in atherosclerosis (33–38). Studies with transgenic mice overexpressing human apoA-I or apoA-II have shown that apoA-I decreases and apoA-II increases atherosclerotic lesion development (39, 40). Variation in the apoA-II content in HDL has also been reported to affect interactions with metabolically important proteins, hepatic lipase, LCAT, and CETP (41–45). These results suggest that the ratio of apoA-I and apoA-II in HDL influences the important protein–protein interactions in HDL and may change its metabolic fate. In this work we report that PLTP interacts with plasma apoA-I and apoA-II, and that the binding region for PLTP on apoA-I resides in the amino terminal domain.

Based on gel filtration chromatography data, CETP and PLTP activities appear to be associated with different HDL subclasses (14, 46). PLTP activity coeluted with a fraction of particles similar in size to human HDL_2 (300–375 kD) (14), which corresponds closely but not completely with the LpA-I fraction (32). In vivo studies demonstrate that PLTP functions optimally in transgenic mice overexpressing human apoA-I (5), a finding also observed for both LCAT (47) and CETP (48). The transgenic animal studies suggest that all these proteins may interact with apoA-I in HDL. The apoA-II/apoA-I molar ratio in the HDL particles is, in fact, an important determinant of PLTP-mediated HDL conversion (13), and PLTP cannot mediate the conversion of particles that lack apoA-I (12, 13, 49). All these results support our finding that PLTP binds to apoA-I.

The structure of human apoA-I has been investigated extensively and been shown to possess multiple tandem-repeating 22-mer amphipathic α-helices. The lipid-binding domains have been assigned to the N- and C-termini. Recently it has been proposed that the C-terminal helices are involved in the initial binding of apoA-I to the lipid surface to form HDL particles (21, 50, 51). A possible interaction between HDL_3 and a putative liver HDL receptor is also reported to be mediated by the C-terminal region of apoA-I (52). We located a PLTP-binding site between amino acids 27–141, and the most effective competitor for PLTP binding to apoA-I in an ELISA assay was a monoclonal anti-apoA-I antibody whose epitope is located between amino acids 27–48. The immunoreactivity of these antibodies is influenced by phospholipid and cholesterol (53, 54), which are both transferred by PLTP (8, 55). The antibodies with C-terminal or the most N-terminal epitopes had no effect on PLTP binding. It was recently reported that CETP can bind to human apoA-I and apoA-II (56, 57), and that the amino terminal regions of apoA-I are also involved in the interaction between HDL and the lipopolysaccharide-binding protein (LBP) (58), both of which share sequence homology with PLTP (57).

Binding of PLTP to the amino terminal portion of purified apoA-I raises some important physiological aspects concerning its binding to apoA-I in human HDL. Although the secondary structure of apoA-I appears similar in all HDL subclasses, several reports show that the microconformation of apoA-I may vary from one particle to another. The differences in apoA-I microconformation may depend on various parameters, including the particle size (60), the number of apoA-I molecules per particle, the number of helical segments in contact with lipid (61, 62), and the content of free cholesterol in HDL (63). These different conformations of apoA-I are probably critically important for the physiological functions of HDL. Thus, the change in conformation of apoA-I that likely occurs in a given HDL particle during its maturation or remodelling might result in a profound change in the reactivity of that particle with PLTP. This reactivity might depend on the exposure of a crucial amino terminal portion of apoA-I on the particle surface. On the other hand, PLTP has been shown to possess high affinity and binding capacity for phosphatidylcholine (55), suggesting that PLTP can, in addition to the amino terminal portion of apoA-I, bind to phospholipid that is associated with apoA-I. Further investigations are necessary to clarify this aspect.

The interaction of PLTP with human apoA-I may be

an important phenomenon involved in reverse cholesterol transport. In addition to phospholipid transfer, the binding of PLTP to the amino terminus of apoA-I may, during PLTP-mediated HDL conversion, be the driving force in release of apoA-I from the HDL particle (12, 22). The released apoA-I has preβ-mobility and contains some phospholipids. If PLTP is also located in the preβ-fraction, interacting with apoA-I after its dissociation, it is possible that this complex acts as an acceptor for peripheral cell cholesterol and/or phospholipid, and that the apoA-I-bound PLTP mediates transfer of these lipids to the acceptor. This kind of PLTP/apoA-I/phospholipid complex having $preβ_1$-mobility may function in the early steps of reverse cholesterol transport. In later steps, a functional lipoprotein unit containing apoA-I, apoD, LCAT, and CETP activities has been described, which may catalyze both cholesterol esterification and cholesteryl ester transfer (46).

PLTP binds to apoA-I and apoA-II, and they both compete effectively with one another, indicating that PLTP contains a common binding region for both apolipoproteins. Without apoA-II monoclonal antibodies, we were unable to investigate the PLTP binding site on apoA-II. The physiological relevance of the interaction between PLTP and apoA-II is a question that remains to be answered.

The PLTP-mediated phospholipid transfer is not influenced by the apolipoprotein composition of HDL, while the interconversion of HDL is dependent on the presence of apoA-I. This may be due to the higher affinity of apoA-II for phospholipids and cholesterol (64), and may explain why PLTP cannot release apoA-II from the HDL particle in a process necessary for the fusion. In conclusion, we have shown that PLTP can bind to the major apolipoproteins of HDL, apoA-I, and apoA-II. This PLTP/apolipoprotein interaction further supports a crucial role of PLTP in HDL metabolism and reverse cholesterol transport.

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