

Pig plasma phospholipid transfer protein facilitates HDL interconversion

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Abstract Phospholipid transfer protein (PLTP) from pig plasma was purified to homogeneity using ultracentrifugation and a combination of hydrophobic-, heparin-Sepharose-, and anti-pig-PLTP-affinity chromatography techniques. The molecular weight of PLTP is 78,000 as estimated by SDS-PAGE and by gel filtration. The effect of pig plasma PLTP on the particle size distribution of either human or pig high density lipoprotein (HDL) was studied by incubating HDL with PLTP. Incubation of human HDL₃ or pig HDL with the highly purified preparations of PLTP induced a conversion of the homogeneous HDL into two main populations of particles. The conversion products were isolated by ultracentrifugation at density 1.21 g/ml. The size changes were evident as analyzed by native gradient gel electrophoresis and by high resolution gel filtration. The diameters of the large and small particles formed were 10.8 nm and 7.6–7.9 nm, respectively. In addition, pig HDL conversion products included a third population of particles with a diameter of 11.5 nm. The degree of conversion was dependent on time and PLTP activity. Neither cholesteryl ester transfer protein nor lecithin:cholesterol acyltransferase activity could be detected in the PLTP preparations. ■ The present study demonstrates that purified pig PLTP can act as a conversion factor: it has the ability to convert HDL into populations of large and small particles. The release of apoA-I is an essential part of the conversion process. — Pussinen, P., M. Jauhiainen, J. Metso, J. Tyynelä, and C. Ehnholm. Pig plasma phospholipid transfer protein facilitates HDL interconversion. *J. Lipid Res.* 1995. 36: 975–985.

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The plasma level of high density lipoprotein (HDL) cholesterol is inversely correlated with the risk for coronary heart disease (CHD) (1–3). However, it is still unknown whether HDL mediates a protective effect because of its role in the reverse cholesterol transport pathway or whether a low HDL cholesterol concentration is an indicator of the accumulation of atherogenic lipoproteins of lower density (4). The role of HDL in reverse cholesterol transport has recently been actively investigated. It has been reported that the initial recipient of peripheral cell cholesterol in plasma is a minor HDL subpopulation consisting of small apoA-I-containing particles which upon electrophoresis have pre β -mobility (5, 6). These

lipoproteins loaded with cellular cholesterol are then converted to spherical HDL and transported to the liver for excretion of the cholesterol as bile acids. However, little is known about the factors that control the distribution of HDL subpopulations.

Human HDL consists of several discrete subpopulations of particles differing in size, density, and composition (7, 8) while pig plasma contains a relatively homogeneous population of HDL that resembles the human HDL₃ subfraction (9, 10). Human HDL contains two main apolipoproteins, apoA-I and apoA-II, while in pig the major HDL protein is apoA-I with no detectable apoA-II (11).

There is increasing evidence that different HDL subpopulations have different metabolic functions (12, 13). Thus, factors that regulate the distribution of HDL subpopulations are potentially important for the understanding of cholesterol metabolism and predisposition to CHD. Several plasma factors have been reported to influence HDL particle size. These include lecithin:cholesterol acyltransferase (14), lipoprotein lipase (15), hepatic lipase (16), cholesteryl ester transfer protein (CETP) (17), and a putative HDL conversion factor (18–20). This converting activity in the process of HDL remodeling has been attributed to CETP in some studies (21, 22). We recently reported that human plasma phospholipid transfer protein (PLTP) can cause interconversion of HDL (23). The same conclusion was made by Tu, Nishida, and Nishida (24). Day et al. (25) recently reported the complete protein and nucleic acid sequence of human PLTP (25).

Abbreviations: PLTP, phospholipid transfer protein; HDL, high density lipoprotein; LDL, low density lipoprotein; CHD, coronary heart disease; CETP, cholesteryl ester transfer protein; PBS, phosphate-buffered saline; PC, phosphatidylcholine; DPPC, dipalmitoyl phosphatidylcholine; LCAT, lecithin:cholesterol acyltransferase.

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They showed that human PLTP displays homology with human CETP (20%), human lipopolysaccharide-binding protein (24%), and human neutrophil bactericidal permeability increasing protein (26%).

Functional CETP is nonexistent or minimal in pig plasma (26–28). Whether this is due to lack of CETP or the presence of an inhibitor protein (29) is still an open question. The existence of HDL conversion activity in pig plasma has been reported (30), but further purification of this factor has not been described. Accordingly, the pig offers an interesting experimental animal model to examine factors that promote the interconversion of HDL. The aim of this study was to isolate pig plasma phospholipid transfer protein (PLTP), and to assess its role in the conversion of high density lipoproteins using both human HDL₃ and pig HDL as substrates.

MATERIALS AND METHODS

Egg phosphatidylcholine was from Sigma; 1-palmitoyl-2-[1-¹⁴C]palmitoyl phosphatidylcholine (DPPC, sp act, 55 mCi/mmol) was from Amersham, UK. Butyl-Toyoppearl 650(M) was from Merck, Germany; CNBr-activated Sepharose CL-4B, Protein A-Sepharose CL-4B, Superose 6 HR gel filtration column, and heparin-Sepharose CL-6B were obtained from Pharmacia, Uppsala, Sweden. Reversed phase C-18 column was from Vydac. Sequencing grade modified trypsin (sp act, 13,000 units/mg protein) was from Promega. Heparin (5000 units/ml) was from Medica, Helsinki, Finland. A monoclonal antibody to CETP, mAb TP1, was a gift from Dr. Y. L. Marcel (Heart Institute, University of Ottawa, Ottawa, Canada). All chemicals were of analytical grade.

Isolation of lipoproteins

Human LDL (1.019–1.05 g/ml) and HDL₃ (1.125–1.21 g/ml), pig LDL (1.020–1.087 g/ml) and HDL (1.087–1.21 g/ml) were isolated by sequential ultracentrifugation using KBr to adjust the density (31). All the lipoprotein fractions were exhaustively dialyzed against Tris-buffered saline (TBS) and stored at 4°C.

Assay of PLTP activity

Phosphatidylcholine (PC) liposomes were prepared as described (32) using 10 μmol of egg PC, 1 μCi of [¹⁴C]DPPC, and 20 nmol butylated hydroxytoluene (BHT). The assays were performed in Eppendorf tubes as described by Speijer et al. (33). Each assay contained HDL₃ (250 μg of protein), liposomes (150 nmol of [¹⁴C]PC-labeled liposomes), sample (4–10 μl), and 10 mM Tris-HCl buffer, pH 7.4, containing 150 mM NaCl and 1 mM EDTA in a final volume of 400 μl. Each series contained blank tubes without sample and tubes with a control plasma sample. The reaction was stopped by the addition of 300 μl 536 mM NaCl, 363 mM MnCl₂, and 52 units of heparin. The tubes were briefly vortexed and after standing for 10 min at room temperature centrifuged for 10 min at 15,000 rpm. The radioactivity was determined from 500 μl of the supernatant.

Purification of PLTP from pig plasma

The lipoprotein-depleted fraction, density >1.21 g/ml, was isolated from 480 ml of pig plasma, that had been stored at –70°C, by ultracentrifugation, made 5 mM with respect to 2-mercaptoethanol and chromatographed on a Butyl-Toyoppearl 650(M) column (5.0 × 15 cm) as described by Ohnishi, Yokoyama, and Yamamoto (34). The fractions with PLTP activity were combined, made 5 mM with 2-mercaptoethanol, and applied to a heparin-Sepharose column (3.0 × 6.0 cm) at a flow rate of 60 ml/h. The column was first extensively washed with the equilibration buffer, 25 mM Tris-HCl, pH 7.4, containing 1 mM EDTA, followed by 25 mM Tris-HCl, pH 7.4, containing 50 mM NaCl and 1 mM EDTA. PLTP was eluted from the column with 1 M NaCl in equilibration buffer. The active fractions were combined and an aliquot was applied on a monoclonal anti-pig-PLTP affinity column (15 mg of anti-pig-PLTP IgG coupled to 1 g of gel matrix) by recycling the sample overnight at a flow rate of 20 ml/h. The column was first washed with PBS followed by 0.1 M glycine-HCl, pH 2.8. The PLTP activity was eluted with 0.5% Triton X-100 in glycine buffer. All purification steps were performed at 10°C. The purification procedure is summarized in Table 1.

TABLE 1. Purification of pig PLTP

Purification Step	Total Protein	Total Activity	Specific Activity	Fold-Purification	Recovery
	mg	μmol/h	μmol/h/mg		%
d > 1.21 g/ml	28,600	626	0.022	1	100
Butyl-Toyoppearl	135	484	3.59	163	77
Heparin-Sepharose	29	324	11.2	509	52
Antibody column ^a	1.40	130	92.9	4223	21

^aOnly an aliquot of the heparin-Sepharose eluate was subjected to the antibody column. The results reported in the table are corrected accordingly.

PLTP peptide analysis

Peptide analysis was performed essentially as described (35). The purified protein was exhaustively dialyzed against water, lyophilized, and subjected to SDS-PAGE under non-reducing conditions. After Coomassie Brilliant Blue-staining, the 78 kD protein band was cut out and washed twice with 250 μ l 0.2 M ammonium bicarbonate containing 50% acetonitrile for 20 min at 30°C. After washing the gel, slices were dried under nitrogen. The slices were rehydrated by adding 6 μ l of digestion buffer (0.2 M ammonium bicarbonate containing 0.02% Tween 20) after which 0.5 μ g of methylated trypsin was added twice at 30-min intervals. Digestion buffer was added until the gel slices were covered. The mixture was incubated for 20 h at 30°C. After incubation the digestion solution was removed and 20 μ l of trifluoroacetic acid was added. The peptides were extracted by adding 150 μ l 60% acetonitrile containing 0.1% trifluoroacetic acid twice at 20-min intervals. As a blank a slice of gel containing no protein was treated similarly. The peptides were separated on a narrow bore reversed phase column (C-18, 2.1 \times 250 mm) attached to an Applied Biosystems HPLC-system using a linear acetonitrile gradient (0–60% acetonitrile in 60 min). Elution of peptides was monitored at 220 nm, the peptides were collected and subjected to sequence analysis on an Applied Biosystems 477A/120A protein sequencer using Edman degradation.

Preparation of monoclonal pig PLTP antibody

Female Balb/c mice were immunized subcutaneously with 100 μ g of partially purified PLTP (containing about 25 μ g of PLTP) with complete Freund's Adjuvant (Gibco). On day 30 they received a similar dose of the antigen with incomplete Freund's Adjuvant. On day 60 the mice were boosted intraperitoneally with 100 μ g of the antigen in PBS and killed 3 days later. The splenic lymphocytes were fused with P3-NS1-Ag4-1 cells (NS-1) (36) essentially as described by Gefer, Margulies, and Scharff (37) and the hybridomas were isolated in HAT medium. Culture supernatants were tested for anti-PLTP antibody by ELISA and by Western blotting. A positive culture was cloned twice by limiting dilutions and passaged in Pristane-treated Balb/c mice for ascites production.

Purification on IgG

The Ig subclass of the antibody was determined by ELISA in which the plates were coated with pig PLTP, incubated with hybridoma culture supernatant, and thereafter with mouse Ig subclass-specific rabbit antisera purchased from Miles Laboratories, Inc. Detection was performed using AFOS-conjugated goat anti-rabbit antisera (Sigma). The monoclonal antibody (IgG₁) was purified from ascites fluid with a Protein A-Sepharose CL-4B according to Harlow and Lane (38).

Incubation of PLTP with human HDL₃ and pig HDL

Incubations were carried out in 10 mM Tris-HCl buffer, pH 7.4, containing 150 mM NaCl. Samples were incubated in Eppendorf tubes in a total volume of 600 μ l for 22–46 h at 37°C in a shaking water bath. After completion of the incubation, the tubes were placed on ice. Control incubations of HDL in the absence or presence of purified PLTP were also carried out at 4°C. The amount of PLTP in the incubation was 200–2,000 nmol/h, and the incubation experiments were performed with PLTP originating from each purification step.

Isolation of HDL conversion products

The density of the incubated sample was adjusted to 1.21 g/ml with solid KBr and the ultracentrifuge tubes were filled up to 3 ml with density solution 1.21 g/ml. The HDL particles were isolated by ultracentrifugation in TL-100.3 angle rotor at 100,000 rpm for 16 h at 10°C in the Optima TL-100 Tabletop Ultracentrifuge (Beckman). After centrifugation the samples were divided into three 1-ml fractions beginning from the top.

Analysis of HDL particle size

HDL particle size was assessed with native gradient gel electrophoresis (8) in self-made 4–26% polyacrylamide gels (8.0 \times 8.0 cm). High molecular weight electrophoresis calibration standards from Pharmacia were used as molecular markers. After staining the gels were scanned with the BioImage System (Millipore Co.). HDL particle size was also analyzed by gel filtration chromatography at room temperature on a Superose 6 HR column that was equilibrated and eluted with 10 mM Tris-HCl, pH 7.4, containing 150 mM NaCl. The column was calibrated with gel filtration standards (Bio-Rad). The flow rate was 0.5 ml/min and the fraction size was 0.5 ml.

Other methods

Protein was determined by the method of Lowry et al. using human serum albumin as standard (39). Cholesterol, triglycerides, and phospholipids were assayed by enzymatic methods (40), apolipoproteins A-I, pig A-I, and A-II by immunoturbidometry (41), and CETP activity as described by Groener, Pelton, and Kostner (42). SDS-PAGE was run by the method of Laemmli (43), and Western blotting was performed as described by Towbin, Staehelin, and Gordon (44). Hepatic lipase and LCAT were assayed as described (45, 46).

RESULTS

Pig PLTP was purified from the lipoprotein-depleted plasma density fraction $d > 1.21$ g/ml using the following chromatographic steps: hydrophobic interaction (Butyl-

Toyopearl 650M), heparin-Sepharose, and monoclonal anti-pig-PLTP affinity chromatography. Details of the purification and the recovery of PLTP from pig plasma are given in Table 1. The elution profiles after hydrophobic interaction chromatography, and heparin-Sepharose affinity chromatography are shown in Fig. 1A and B.

In the hydrophobic interaction chromatography (Fig. 1A) 50–80% of PLTP eluted with 3 mM Tris-HCl. Part of

the PLTP was tightly bound to the hydrophobic matrix and could only be eluted with water. This tightly bound PLTP-fraction was unstable during further purification (data not shown) and was therefore not further processed. The advantage of this step was the high recovery of active PLTP (fractions 128–158) that represented only about 0.5% of the protein originally applied to the column. The active fractions were applied on a heparin-Sepharose

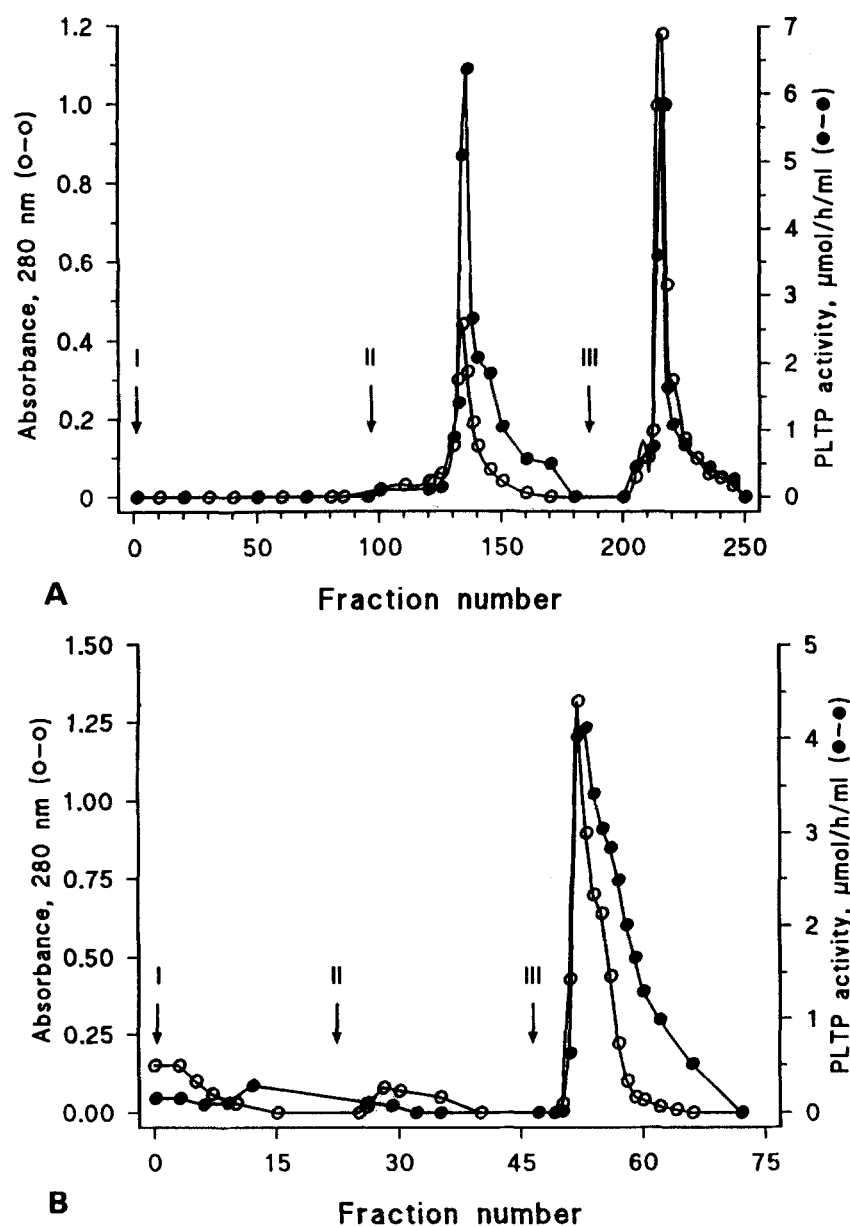


Fig. 1. Purification of pig phospholipid transfer protein. A: Hydrophobic interaction chromatography. The lipoprotein-depleted fraction of pig plasma, $d > 1.21 \text{ g/ml}$, was made 5 mM with respect to 2-mercaptoethanol and chromatographed on a Butyl-Toyopearl 650(M) column. I) After washing with 50 mM Tris-HCl, pH 7.4, 1 mM EDTA, PLTP was eluted with a linear gradient of Tris (50–3 mM). II) The elution was continued with 3 mM Tris-HCl. III) Finally, the column was eluted with purified water. B: Heparin-Sepharose chromatography. PLTP-active fractions eluted with 3 mM Tris-HCl from the hydrophobic interaction chromatography were applied on a heparin-Sepharose column. After washing (I and II), bound proteins were eluted with 25 mM Tris-HCl, pH 7.4, 1 M NaCl, 1 mM EDTA at a flow rate 60 ml/h (III).

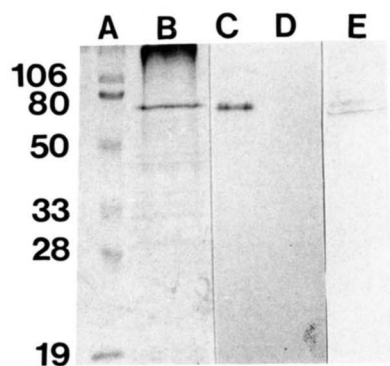


Fig. 2. 12.5% SDS-PAGE of the purified pig plasma PLTP. Lane A. Molecular weight standard. After the antibody column pig PLTP was run in 12.5% SDS-PAGE and detected by silver-staining (lane B), immunoblotting with monoclonal anti-pig-PLTP (lane C), and immunoblotting with monoclonal anti-human-CETP (lane D). Lane E shows immunoblotting of a positive human CETP control (TP-1).

column (Fig. 1B) to which 85–90% of the applied PLTP activity was bound. An aliquot of the fractions (51–67) that eluted from the heparin-Sepharose column with 1 M NaCl were chromatographed on a monoclonal antibody column for pig PLTP. The activity could not be eluted at a low pH, and a detergent was needed to elute the activity. The recovered activity from the column was 40% which represented 5% of the original protein. The antibody column was highly specific for pig PLTP but the capacity

of the column was rather low. Each step of the PLTP purification protocol was reproducible, i.e., in different purifications specific activity, fold-purification and percentage recovery of individual chromatographic steps were highly repeatable. The entire procedure could be completed in 2 weeks.

SDS-PAGE of the active PLTP fractions under reducing conditions revealed a major protein band with a molecular weight of about 78,000, which could also be detected with monoclonal anti-pig-PLTP (Fig. 2). Cholesteryl ester transfer protein (CETP) activity could not be detected in any of the Butyl-Toyopearl column fractions or in the pooled fractions with high PLTP activity after heparin-Sepharose or the antibody column steps. Nor did immunoblotting with polyclonal or monoclonal anti-human-CETP (TP1), detect any CETP protein (Fig. 2). During purification, hepatic lipase and LCAT activities were measured. After the heparin-Sepharose step neither of them could be detected.

The purified pig PLTP protein was digested with trypsin and peptides were isolated and sequenced. Four of the peptides and the complete protein sequence of human PLTP (25) are shown in Fig. 3. These preliminary data suggest that a high homology may exist between these two proteins.

To assess the effect of pig PLTP on HDL conversion, human HDL₃ and pig HDL were incubated in the presence of purified PLTP for 22–46 h at 37°C. After incubation the HDL particles were isolated by ultracentrifugation.

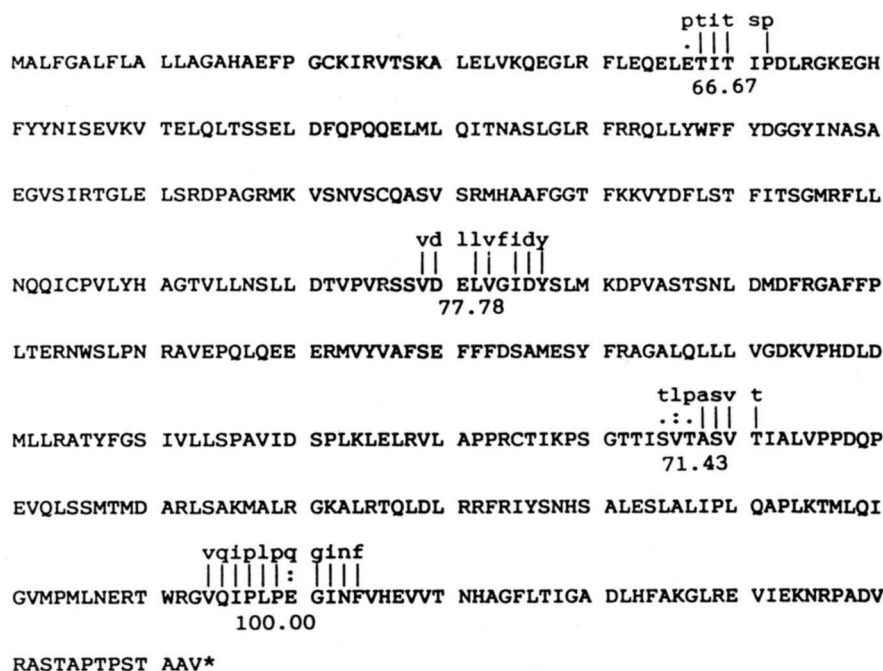


Fig. 3. Comparison of pig PLTP peptide sequences to human plasma PLTP protein sequence (25). The comparison was performed using the GCG program BESTFIT. The pig PLTP peptides are denoted by lower case letters and the human protein sequence by upper case letters. The percent similarity is indicated under the respective sequences.

trifugation at 1.21 g/ml. HDL particle size was determined by native gradient gel electrophoresis (**Fig. 4A and B**) and by high resolution gel filtration on Superose 6HR (**Fig. 5A and B**). The chemical compositions of human HDL₃ and pig HDL are shown in **Table 2**. The human

HDL₃ was homogeneous with a mean particle diameter of 8.7 nm. Pig HDL consisted of a homogeneous population of particles with a mean particle diameter of 9.0 nm. When the HDL were incubated in the absence of PLTP at 37°C or in the presence of PLTP at 4°C the particle

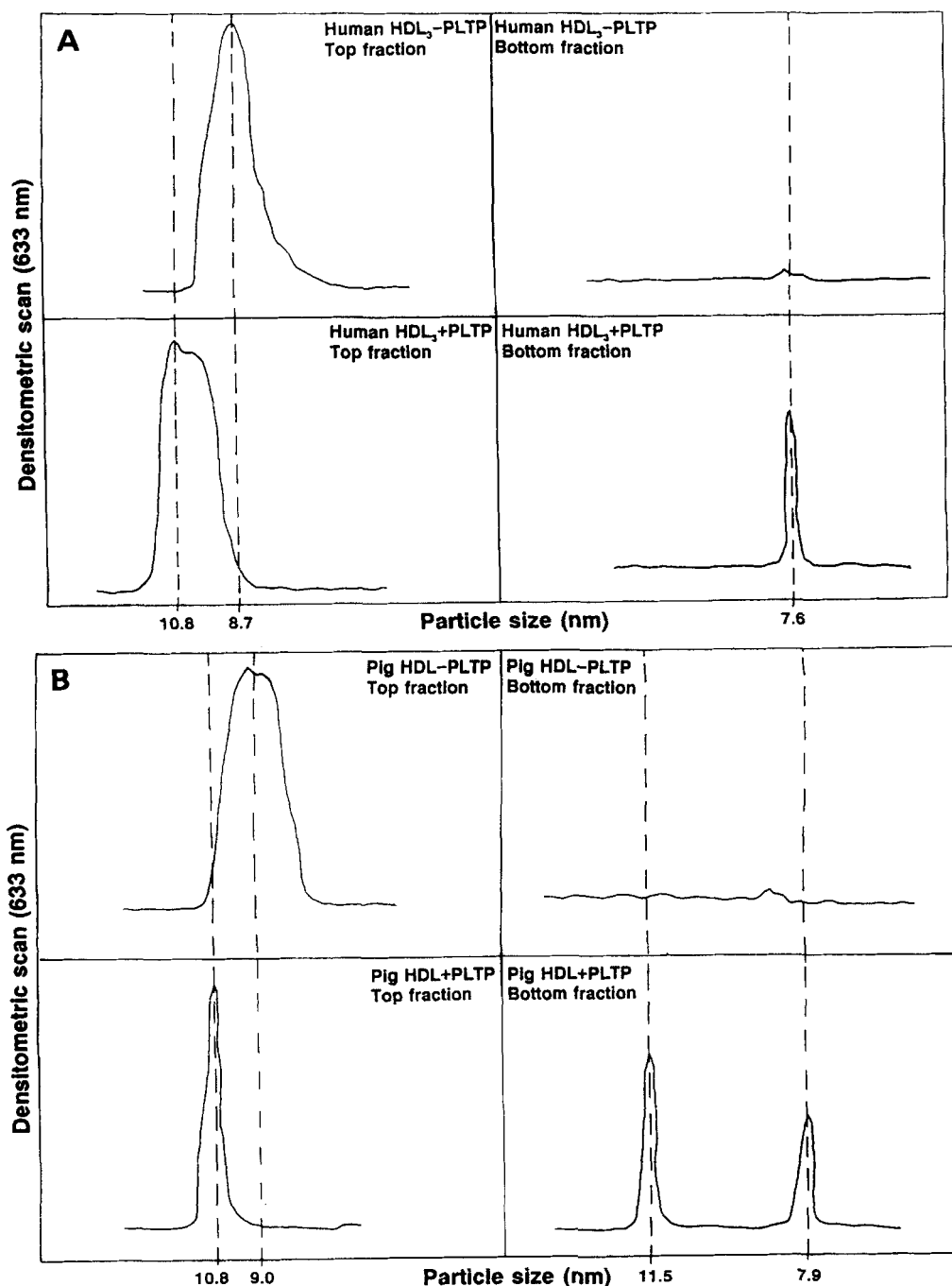


Fig. 4. Determination of HDL particle size by native gradient gel electrophoresis after ultracentrifugation at d 1.21 g/ml. Human HDL₃ (A) or pig HDL (B) (0.8 mg of protein) were incubated for 46 h at 37°C in the absence or presence of purified PLTP (700 nmol/h), final volume 600 μ l. After incubation, 500 μ l of the incubated mixture was adjusted to d 1.21 g/ml and ultracentrifuged for 16 h at 100,000 rpm in a final volume of 3 ml. After centrifugation the tube was divided into three equal volume fractions (1 ml) from the top and 30 μ g protein was mixed with the sample buffer and applied on a gel. After electrophoresis the gels were scanned as described in Materials and Methods. The results were similar when the experiments were performed with either pig or human PLTP.

size remained unchanged. When increasing amounts of PLTP (activity from 200 to 2,000 nmol/h) were added to the incubation mixture, changes in particle size were evident. Both gradient gel electrophoresis (Fig. 4A and B) and gel filtration (Fig. 5A and B) demonstrated that the original HDL was converted into populations of large and

small particles. The large particles derived from both human and pig HDL had a diameter of 10.8 nm and the small particles had diameters of 7.6–7.9 nm in human HDL and 7.9 nm in pig HDL. After ultracentrifugation, the large particles floated in the top fraction, while the small particles could be recovered in the bottom fraction.

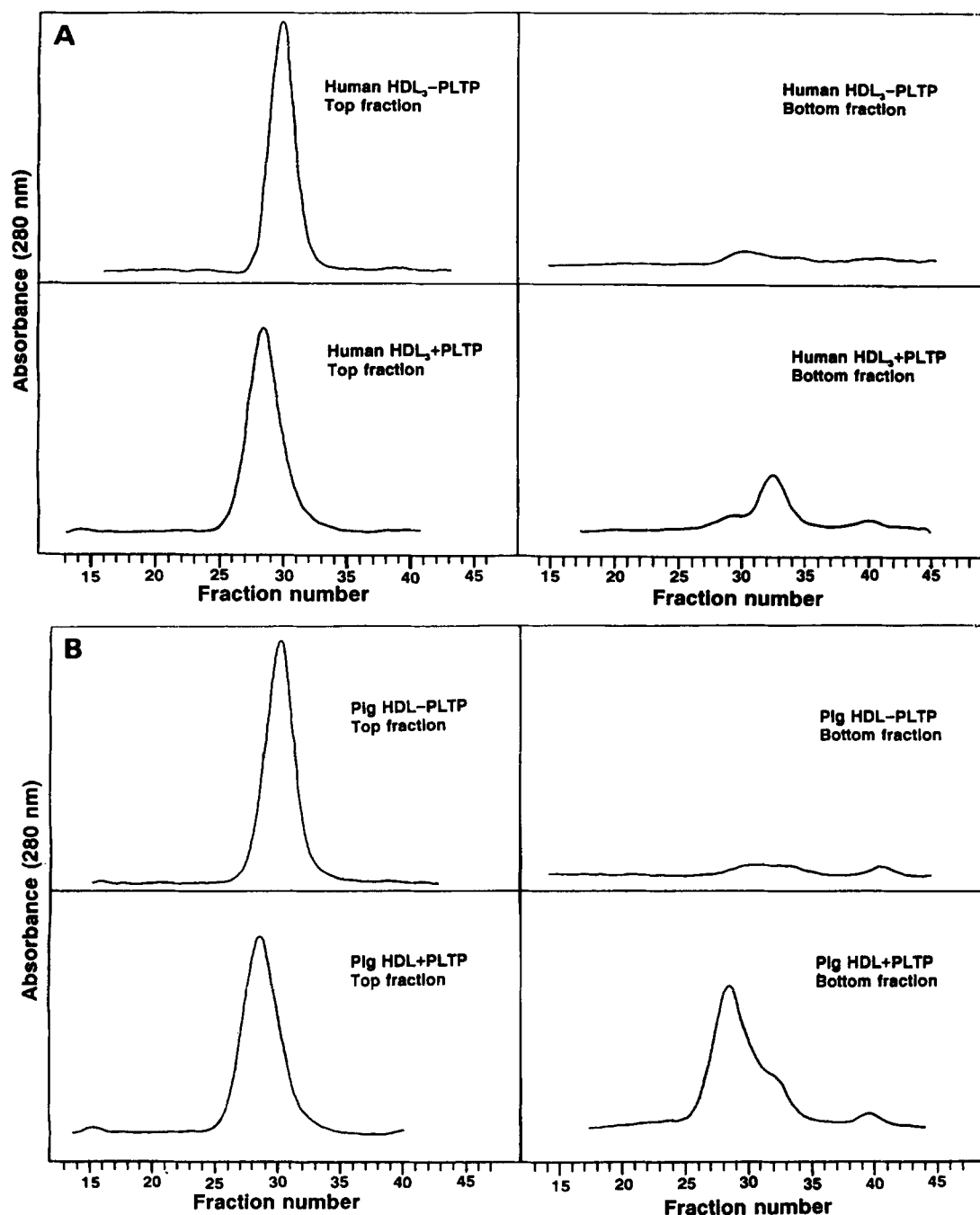


Fig. 5. Determination of HDL particle size by high resolution gel filtration on a Superose 6 HR after ultracentrifugation at d 1.21 g/ml. Human HDL₃ (A) or pig HDL (B) (0.8 mg of protein) were incubated for 46 h at 37°C in the absence or presence of purified PLTP (700 nmol/h), final volume 600 μ l. After ultracentrifugation, a 600- μ l sample of each fraction was applied on a Superose 6 HR column and eluted with 10 mM Tris-HCl, pH 7.4, 150 mM NaCl, followed by A_{280} measurements of the fractions. The results were similar with pig and human PLTP.

TABLE 2. Chemical composition of human HDL₃ and pig HDL before and after incubation with purified pig PLTP

Lipoprotein	Composition				Particle Size
	Protein	PL	Chol	TG	
		mass %			nm
Human HDL ₃ ^a	58.3	26.8	12.7	2.2	8.7
Large particles ^b	56.9	27.8	11.8	3.5	10.8
Small particles ^c	88.5	10.4	1.0	—	7.6–7.9
Pig HDL ^a	52.2	31.2	14.5	2.1	9.0
Large particles ^b	50.0	32.5	14.0	3.5	10.8
Small particles ^c	86.9	11.9	1.1	—	7.9
Large particles ^d	86.6	12.2	1.2	trace	11.5

^aHuman HDL₃ or pig HDL was incubated as a control sample without PLTP at 37°C for 46 h and ultracentrifuged at density 1.21 g/ml.

^bHuman HDL₃ or pig HDL was incubated with PLTP at 37°C for 46 h and ultracentrifuged at d 1.21 g/ml. After spin these particles were in the top fraction.

^cAfter centrifugation these particles were in the bottom fraction. Details are in Materials and Methods and in the legend of Fig. 5.

The 1-ml medium fraction contained no detectable lipoprotein. Pig HDL displayed, after PLTP incubation, an additional population of particles with $d > 1.21$ g/ml. The diameter of these particles was 11.5 nm. After a 46-h incubation the original human and pig HDL populations were almost completely converted into large and small HDL particles.

The PLTP-dependent HDL particle conversion was also monitored using gel filtration on a Superose 6 HR column after the particles had been isolated by ultracentrifugation at 1.21 g/ml. Without PLTP treatment human HDL₃ eluted at a position corresponding to a molecular weight of 170,000 and pig HDL at a position of 180,000. The new populations of large and small particles derived from human and pig HDL upon incubation with PLTP had apparent molecular weights of 220,000 and 44,000, respectively. The chemical compositions of these novel HDL populations were determined (Table 2). They turned out to be highly similar in the corresponding human and pig particles formed. It is noteworthy that the small particles (mol wt 44,000) derived from human HDL₃ or pig HDL consisted mainly of apoA-I and phospholipids. The third novel population derived from pig HDL corresponded to a molecular weight range of 250,000–260,000. These particles consisted mainly of apoA-I and phospholipids with a trace of free cholesterol and triglycerides. The interconversion of both HDL increased progressively as a function of incubation time (data not shown).

The protein recoveries in the ultracentrifugation and the gel filtration steps after the HDL interconversion reaction were calculated. The total protein recoveries after these two procedures ranged between 72 and 86%. In the gel filtration the protein recovery was usually 88–99%.

When a sample of human HDL₃ or pig HDL that had been incubated in the absence of PLTP was ultracentrifuged, 7.6–9.9% of the HDL protein was in the bottom fraction with $d > 1.21$ g/ml. PLTP treatment released apoA-I from the original HDL to the bottom fraction. In the HDL samples incubated with PLTP the protein distributions in the top and bottom fractions were 80.9 and 19.1% in human HDL₃ and 70.1 and 29.9% in pig HDL, respectively (1 mg of HDL protein, 1.0 μ mol/h PLTP, 46 h). Therefore, the PLTP-mediated net protein release in this case in the bottom fraction was about 10% for human HDL₃ and 20% for pig HDL. The protein derived from the added PLTP preparation represents less than 2% of the total protein mass in the incubation mixture. The release of apoA-I from HDL was dependent on the amount of added PLTP activity, and the amount of apoA-I increased in the bottom fraction while it decreased in the top fraction (Fig. 6). Concomitantly, the size of the particles in the top fraction increased.

The phospholipid recoveries ranged between 62 and 76% after ultracentrifugation and gel filtration. Analysis of phospholipid distribution showed that there was about 3.3–4.7% of phospholipid in the bottom fraction after PLTP incubation, whereas in the absence of PLTP less than 1% of total phospholipid was in this fraction.

To determine whether other lipoproteins had an effect on the HDL conversion process, experiments were also performed in the presence of human or pig LDL (protein mass ratio, LDL/HDL = 1). After incubation with PLTP for 46 h at 37°C, both human HDL₃ and pig HDL were converted into distinct particle populations in the same way as in the absence of LDL. On the other hand, PLTP had no effect on LDL particle size whether incubated in the presence or absence of HDL (data not shown).

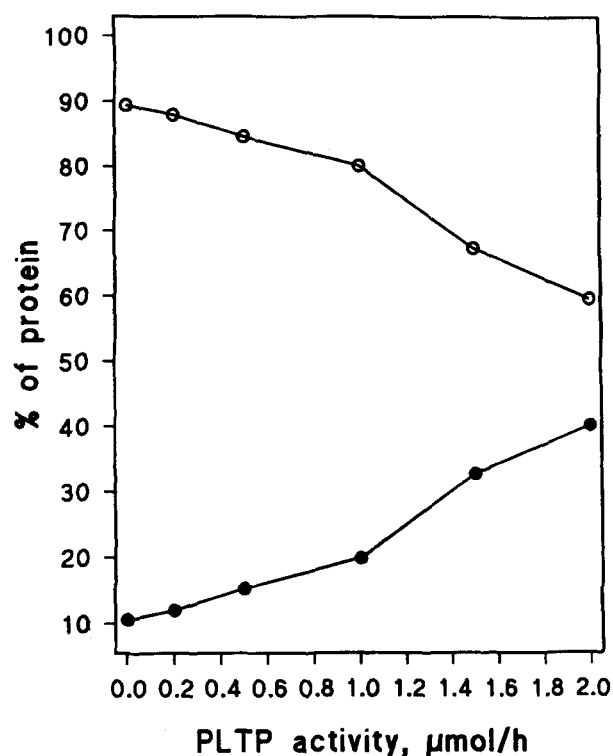


Fig. 6. Effect of PLTP concentration on the distribution of protein in the top and bottom fractions after ultracentrifugation. Pig HDL (1 mg of protein) was incubated with increasing PLTP concentrations for 46 h at 37°C. After incubation the density of the samples was adjusted to 1.21 g/ml with solid KBr and the samples were ultracentrifuged in a final volume of 3 ml (see Materials and Methods). After centrifugation the samples were divided into three equal volume fractions (1 ml each) beginning from the top. The protein (pig apoA-I) distribution in the top and bottom fractions was determined; (○) top fraction; (●) bottom fraction.

To compare the interconversion effects of pig PLTP and human PLTP, the HDL conversion experiments were also performed with human PLTP isolated from lipoprotein deficient plasma. In both human HDL₃ and pig HDL preparations, the human PLTP induced a conversion similar to that obtained with pig PLTP (Figs. 4 and 5). This interconversion was dependent on time and the activity human PLTP (data not shown).

DISCUSSION

The pig phospholipid transfer protein described for the first time in this study has an apparent molecular weight of 78,000 and facilitates the transfer of radiolabeled phosphatidylcholine (PC) from PC-liposomes to HDL₃. In these respects it is similar to the PLTP previously purified from human plasma (23, 24). Recent data from Day et al. (25) reported human PLTP cDNA and protein structure. Human PLTP has an apparent molecular weight of 81,000 and a calculated molecular weight of 51,000. The

close similarity of the peptides sequenced from pig PLTP and the corresponding human peptides corroborates the high degree of homology between the two PLTP proteins. The two proteins are also similar immunologically, thus our polyclonal antiserum against human PLTP cross-reacts with pig PLTP in Western blot analysis (data not shown). There are, however, also differences between the proteins as our monoclonal anti-pig-PLTP antibody recognizes only weakly the human protein.

The pig has been considered an attractive animal model for atherosclerosis research. Interest in the pig stems from the fact that it develops spontaneous and experimentally induced atherosclerotic lesions that are pathologically similar to those found in humans (47). However, there are several differences between the pig and the human lipoprotein systems. These include heterogeneity of the buoyant density of LDL (9), the extensive apoB polymorphism including eight apoB alleles (48), and the quantity of apoA-II (11). Our preliminary results have confirmed that apoA-II is nearly absent in the pig. Pig HDL consists mainly of a homogeneous population of particles with a density and size similar to those of human HDL₃. Of interest, the pig has no detectable cholesteryl ester transfer protein (CETP) (26, 27) or its activity is inhibited by lipid transfer inhibitor protein (LTIP) (28). Although controversial data on the existence of cholesteryl ester transfer activity in pig plasma has been reported (29), this study suggests that pig plasma is devoid of CETP activity but that a putative HDL conversion factor (30), here called PLTP, exists in pig plasma. These observations provide a convenient model for testing the function of purified pig PLTP on HDL interconversion.

The conversion activity of pig PLTP reported here is similar to that of human PLTP that we and others have described recently (23, 24). Moreover, pig PLTP has the ability to convert both pig HDL and human HDL₃. Also, human PLTP can mediate the interconversion of both pig and human HDL to large and small particles.

The present study clearly demonstrates that pig PLTP can act as an HDL conversion factor *in vitro*. It converts a single population of HDL into novel subpopulations of particles with larger and smaller diameters than the original one. This capacity to produce both large and small HDL particles simultaneously is a characteristic feature of PLTP and different from that of several other plasma factors known to influence the density and size of HDL. The conversion activity described here is abolished upon incubation at 58°C for 1 h, a feature attributed to PLTP (49). This fact differentiates it from the HDL conversion factor reported earlier by Barter et al. (20).

The newly formed large particles in the top fraction have the same diameter as naturally occurring human HDL₂ which is thought to be quickly taken up by putative liver receptors (50, 51). Incubation of pig HDL with PLTP repeatedly produced two types of large particles,

whereas in the case of human HDL₃ only one major population of large particles was observed after PLTP function. The composition of the second type of large pig HDL particles in the bottom fraction was very similar to that of the small particles, i.e., they were highly enriched in apoA-I. The large particle size may be due to the existence of several apoA-I molecules per particle. Another explanation is the absence of high affinity apoA-II in the pig which could cause the different distribution of conversion products.

Processes promoting the formation of very small HDL particles may play an important role in the reverse cholesterol transport pathway. Our data agree with the recent observations where the process of PLTP-mediated HDL enlargement was accompanied by the release primarily of apoA-I (24). The small conversion products observed in this study resemble the population of small pre β -HDL in their chemical composition (5). The small HDL is thought to act as the first acceptor of unesterified cholesterol from the peripheral cell surface. The cholesterol-loaded particles may also provide a continuing supply of small HDL functioning as substrate for LCAT (6). Our results thus arouse the intriguing possibility that PLTP might participate in the reverse cholesterol transport by regenerating small HDL particles.

Considering the results presented here, it is surprising that HDL₂ is not found in pig plasma. This may be due to the rapid catabolic rate of pig HDL. The $t_{1/2}$ of pig HDL is approximately 8 h (52). It may well be that under our in vitro conditions the population of pig HDL particles resembling human HDL₂ can be detected because there is no clearing system present. On the other hand, the absence of apoA-II in the pig may cause differences in the behavior of HDL particles compared to other species. The presence of apoA-II in HDL correlates with the classification of HDL into populations differing in size, density, and composition. This study suggests that apoA-II is not necessary in the PLTP-mediated HDL interconversion. The detailed mechanism of this interconversion process and its physiological significance remain to be resolved. ■

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