Phospholipid Transfer Is a Prerequisite for PLTP-Mediated HDL Conversion†

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ABSTRACT: Phospholipid transfer protein (PLTP) is an important regulator of high-density lipoprotein (HDL) metabolism. The two main functions of PLTP are transfer of phospholipids between lipoprotein particles and modulation of HDL size and composition in a process called HDL conversion. These PLTP-mediated processes are physiologically important in the transfer of surface remnants from lipolyzed triglyceride-rich lipoproteins to nascent HDL particles and in the generation of preβ-HDL, the initial acceptor of excess peripheral cell cholesterol. The aim of the study presented here was to investigate the interrelationship between the two functions of PLTP. Plasma PLTP was chemically modified using diethylpyrocarbonate or ethylmercurithiosalicylate. The modified proteins displayed a dose-dependent decrease in phospholipid transfer activity and a parallel decrease in the ability to cause HDL conversion. Two recombinant PLTP mutant proteins, defective in phospholipid transfer activity due to a mutation in the N-terminal lipid-binding pocket, were produced, isolated, and incubated together with radioactively labeled HDL_3. HDL conversion was analyzed using three methods: native gradient gel electrophoresis, ultracentrifugation, and crossed immunoelectrophoresis. The results demonstrate that the mutant proteins (i) are able to induce only a modest increase in HDL particle size compared to the wild-type protein, (ii) are unable to release apoA-I from HDL_3, and (iii) do not generate preβ-mobile particles following incubation with HDL_3. These data suggest that phospholipid transfer is a prerequisite for HDL conversion and demonstrate the close interrelationship between the two main activities of PLTP.

The ability of high-density lipoproteins (HDLs)[1] to protect against the development of atherosclerotic coronary artery disease (CAD) has been well documented and is thought to be due to their role in reverse cholesterol transport (RCT). According to this hypothesis, HDL removes cholesterol from peripheral cells and transports it to the liver for excretion (*1*). Particular subfractions of HDL, such as the preβ-HDL fraction, may be especially efficient in mediating cholesterol removal from peripheral cells (*2*). The HDL in human plasma consists of several subpopulations of particles with distinct composition, structure, and function. This heterogeneity, resulting from continuous remodeling of HDL by plasma factors, has important implications in terms of the cardioprotective functions of HDL. Proteins which are involved in the regulation of HDL subclass distribution include lecithin-cholesterol acyltransferase (LCAT), phospholipid transfer protein (PLTP), and cholesteryl ester transfer protein (CETP). Together with hepatic lipase (HL) and lipoprotein lipase (LPL), these factors modulate the structure of HDL.

Phospholipid transfer protein is a plasma glycoprotein that plays an important role in HDL metabolism (*3*−*6*). The two main functions attributed to PLTP are transfer of phospholipids and size conversion of HDL particles. PLTP is capable of transferring phospholipids between lipoprotein classes, the most effective transfer occurring between HDL subpopulations (*7*) and between cell membranes and lipoproteins (*8*). The physiological significance of PLTP in vivo was convincingly demonstrated in a knockout mouse model. Plasma of the $PLTP^{-/-}$ animals displayed an almost total lack of PL transfer activity, and the HDL levels were drastically reduced (*6*). When fed a high-fat diet, the mice accumulated surface remnants of triglyceride-rich lipoproteins. The PLTP-mediated PL transport thus appears to be crucial in the transfer of the lipolytic surface remnants to lipid-poor apoA-I particles, to maintain physiological levels of plasma HDL (*6*). PLTP is also capable of transforming HDL_3 into large HDL particles with a concomitant release of preβ-mobile lipid-poor apoA-I (*9*, *10*). This PLTP-mediated conversion process is physiologically relevant as it increases plasma preβ-HDL levels (*11*, *12*).

Despite several reports about the two activities of PLTP, their exact mechanisms and interrelationship are still not well characterized. PLTP acts as a nonspecific lipid transfer protein, being capable of transferring several phospholipid classes (*7*, *13*). Molecular modeling and site-directed mutagenesis of the protein have provided evidence for the

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[1] Abbreviations: HDL, high-density lipoprotein; PLTP, phospholipid transfer protein; CETP, cholesteryl ester transfer protein; LCAT, lecithin-cholesterol acyltransferase; apoA-I, apolipoprotein A-I; rPLTP, recombinant PLTP; pPLTP, plasma PLTP; PL, phospholipid; DEPC, diethylpyrocarbonate; Thimerosal, ethylmercurithiosalicylate; GGE, gradient gel electrophoresis.

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presence of two lipid-binding pockets in the molecule and for their involvement in the PL transfer reaction (*14*). Particle fusion has been shown to be the mechanism of PLTP-mediated HDL conversion (*15*, *16*). HDL conversion is influenced by the surface apolipoproteins and by the core lipid composition of the particles (*17*, *18*). In the study presented here, we have investigated the relationship between the PLTP-mediated phospholipid transfer and HDL conversion. Results obtained using specific chemical inhibition of PLTP-mediated PL transfer and mutant proteins with defects in the N-terminal lipid-binding pocket demonstrate that phospholipid transfer is a prerequisite for efficient PLTP-mediated HDL conversion.

EXPERIMENTAL PROCEDURES

Purification of Plasma PLTP. PLTP was purified from fresh human plasma essentially as described previously (*19*). The only modification to the published procedure was the use of a 1 to 100 mM sodium phosphate buffer (pH 6.8) gradient in the final hydroxyapatite chromatographic purification step. The phospholipid transfer activity of the purified plasma PLTP (pPLTP) was 6000−8000 nmol mL^{-1} h^{-1}. The assay method was based on assessment of the transfer of phosphatidylcholine from [^{14}C]DPPC-labeled PC liposomes to HDL_3 (*9*, *20*). The protein appeared as a single major 80 kDa band on SDS−PAGE by silver staining.

Production and Purification of Recombinant PLTP. A hexahistidine tag was engineered into the C-terminus of full-length human PLTP cDNA using PCR. The modified cDNA was cloned into pBluescript (Stratagene), and mutations F196W and F464E were introduced using the QuickChange Site-Directed Mutagenesis Kit (Stratagene) according to the manufacturer's instructions. The entire cDNA sequence was verified by dideoxy sequencing using the Sequenase 2.0 Kit (United States Biochemicals). The cDNAs were transferred into the baculovirus expression vector pVL1393 (Pharmingen), and virus stocks and recombinant proteins were produced as described previously (*21*).

Recombinant PLTP was isolated using two chromatographic steps. Briefly, 50 mL of culture supernatant from infected Sf-9 cells was centrifuged for 5 min at 500*g* to remove detached cells. The supernatant was applied onto a 5 mL Hi-Trap Heparin Sepharose column (Pharmacia) at a rate of 1 mL/min at room temperature; the column was washed with 25 mM Tris-HCl buffer (pH 8.0), and the bound protein was eluted with 2 M NaCl in the buffer described above. The fractions containing PLTP (total 8 mL) were diluted 1:1 with 100 mM sodium phosphate (pH 8.0), and the solution was circulated overnight through a column containing 3 mL of Ni−NTA Superflow Agarose (Qiagen) at 4 °C. The column was first washed at room temperature with 50 mM sodium phosphate and 300 mM NaCl (pH 8.0) and then with the buffer supplemented with 20 mM imidazole. The bound PLTP was eluted with 200 mM imidazole in the same buffer. The protein-containing fractions (total volume of 5 mL) were combined and dialyzed extensively against TBS [10 mM Tris-HCl and 150 mM NaCl (pH 7.4)].

Incubation of PLTP in the Presence of Chemical Inhibitors. A stock solution (400 mM) of diethyl pyrocarbonate (DEPC, Sigma) was freshly prepared in anhydrous ethanol. PLTP was incubated in the presence of 0.25−10 mM DEPC for 30 min at 25 °C. Control tubes contained PLTP and the same final volume of ethanol (5% v/v) that was used with each inhibitor concentration. After dialysis against TBS, the phospholipid transfer and conversion activities were determined.

A stock solution (200 mM) of ethylmercurithiosalicylate (Thimerosal, Pally) was prepared in TBS. PLTP was incubated in the presence of 10−100 mM Thimerosal for 30 min at 25 °C. After dialysis against TBS, the phospholipid transfer and conversion activities were determined.

Determination of PLTP Mass. To reliably compare the function of the wild-type (wt) and mutant rPLTP proteins, we isolated the mutant proteins in parallel with a reference wt preparation and started the HDL conversion experiment within 6 h of purification of the proteins. To equalize the amount of wt and mutant proteins used in the conversion experiments, a rapid mass assay method employing Ni−NTA HisSorb Plates (Qiagen) was established. The isolated proteins were diluted to a final volume of 200 μL with PBS and incubated on the plates for 2 h at 37 °C under gentle (400 rpm) shaking. After the wells had been washed six times with PBS on a Labsystems Multiwash apparatus, 3 μg of polyclonal anti-PLTP antibody R164 (*22*) was added and incubated in the wells for 1 h (37 °C). Unbound antibody was washed as described above, and secondary horseradish peroxidase-conjugated goat anti-rabbit IgG (Bio-Rad) was applied and incubated as described above. Formation of the *o*-phenylenediamine chromogenic product was assessed at A_{492}. The antibodies were diluted with a 0.5% BSA/PBS mixture. Increasing amounts of isolated PLTP (5, 10, and 25 μL) applied onto Ni−NTA HisSorb plates resulted in a linear response ($r = 0.99$) after quantitation of the bound protein with the anti-PLTP antibody. The protein mass of PLTP was determined using a sandwich ELISA (*23*).

Measurement of the Extent of HDL Conversion. The conversion experiments were carried out by incubating HDL_3 (250 μg of total protein, final incubation volume of 1 mL) in the presence or absence of PLTP in TBS buffer at 37 °C for ≤48 h. The amount of both wt and mutant rPLTP used in the conversion experiments was 10 μg/incubation. Following incubation, three different methods were employed to determine the extent of HDL conversion. The change in particle diameter was determined using nondenaturing gradient gel electrophoresis (GGE) on self-made 4 to 30% gels (*24*). After incubation, 6 μg of HDL protein was electrophoresed for 20 h at 150 V and 4 °C. The gels were analyzed using the Kodak EDAS120 imaging system and the Kodak Digital Science 1D software. The diameters of the observed particles were determined by comparing their mobilities to that of a high-molecular weight standard (Pharmacia).

To quantitate the released apoA-I from HDL_3, a sensitive radioactive method was established: HDL_3 was radioactively labeled with ^{35}S-labeling reagent (Amersham) (*19*). After the conversion incubation, the density of the reaction mixture was adjusted to 1.21 g/mL with KBr and the samples were centrifuged at 550000*g* for 18 h at 5 °C in a Beckman Optima TL ultracentrifuge (*25*). After centrifugation, 200 μL fractions were collected starting from the top and the amount of radioactivity was determined (1219 Rackbeta, Wallac). The amount of radioactivity in the five bottom fractions was taken to represent the released apoA-I and is presented relative to the total amount of radioactivity. In the bottom fractions,

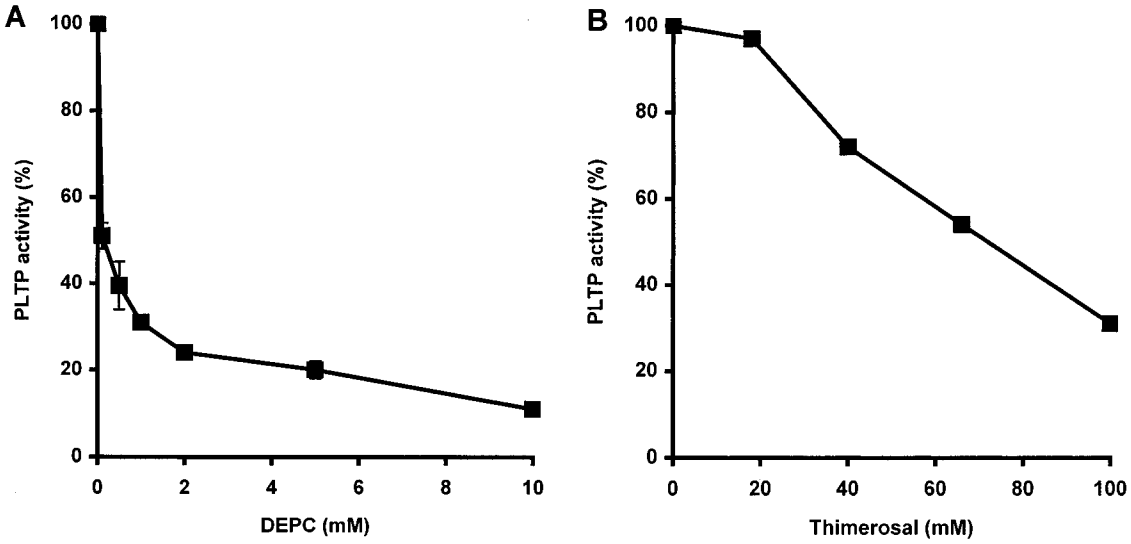

FIGURE 1: Effect of chemical modification on PLTP-mediated phospholipid transfer. Purified plasma PLTP was incubated in the presence of increasing concentrations of (A) diethylpyrocarbonate or (B) ethylmercurithiosalicylate for 30 min at 25 °C. The samples were then dialyzed, and the phospholipid transfer activity was determined using a radiometric assay. The activity of the noninhibited PLTP is presented as 100%. Results from representative experiments with both inhibitors are shown.

apoA-I was the only apoprotein recovered after ultracentrifugation.

To determine the mobility and quantity of the released apoA-I, crossed immunoelectrophoresis (*26*) was performed. Briefly, 1.5 μg of protein from the conversion mixture was first run in a 1% agarose (Bio-Rad) gel in 50 mM barbital buffer (pH 8.6) for 90 min at 250 V. The second dimension was electrophoresed in 1% agarose gels containing 1.25% (v/v) polyclonal anti-apoA-I antiserum for 20 h at 50 V. After removal of salts, the gels were dried and stained for 5 min with 0.5% Serva R-250 Blue (Serva) in a 45% ethanol/10% acetic acid mixture. Control HDL_3 in 0.5% bromophenol blue (Merck) was run in parallel in the second dimension.

RESULTS

Use of Chemically Modified PLTP To Study PL Transfer and HDL Conversion. To study whether the PLTP-mediated PL transfer and HDL conversion are interconnected, we employed pretreatment of PLTP with covalent modifying agents. On the basis of screens performed in our laboratory and previously published data (*27*), diethylpyrocarbonate (DEPC) and ethylmercurithiosalicylate (Thimerosal) were chosen. DEPC modifies histidine residues to form *N*-carbethoxyhistidyl derivatives (*28*), while the other agent that was used, Thimerosal, specifically alkylates free cysteine SH groups.

Incubation of pPLTP with increasing concentrations of DEPC resulted in a dose-dependent inhibition of PL transfer activity (Figure 1A). At 1−2 mM DEPC, 70−80% inhibition was observed. Also, Thimerosal caused a dose-dependent inhibition of the PL transfer activity (Figure 1B). However, compared to that of DEPC, higher concentrations were required. The PL transfer activity of baculovirus-produced rPLTP was inhibited in a similar fashion (data not shown).

To study the effect of these inhibitors on HDL conversion, we incubated HDL_3 in the presence of the chemically modified PLTP preparations. GGE analysis of these HDL_3 conversion experiments is shown in Figure 2. A 24 h incubation of HDL in the presence of native pPLTP induced an increase in the diameter of HDL from 8.8 to 10.4 nm, with concomitant release of small lipid-poor apoA-I, as previously reported (*9, 10*). Incubation of HDL_3 in the presence of PLTP pretreated with the histidine modifier DEPC revealed a concentration-dependent inhibition in the conversion process (Figure 2A). With increasing concentrations of DEPC, the mean diameter of the particles recovered after incubation gradually decreased. At 10 mM DEPC, a concentration causing 90% inhibition of the PL transfer activity, no increase in HDL particle size or release of lipid-poor apoA-I was observed. Similar concentration-dependent inhibition of HDL conversion was observed following incubation of HDL_3 in the presence of Thimerosal-treated PLTP (Figure 2B). Like inhibition of PL transfer activity, relatively high Thimerosal concentrations were required to inhibit HDL conversion. The similar effects of the modifying agents on the two activities of PLTP, PL transfer and HDL conversion, strongly suggested that these two processes are interrelated.

Use of PLTP Mutants To Study PL Transfer and HDL Conversion. To further study the relationship between PLTP-mediated PL transfer and HDL conversion, we used rPLTP proteins carrying point mutations that specifically block the PL transfer activity of PLTP (*14*). The mutant proteins that were chosen, L196W and F464E, which both display severely reduced PL transfer activity, are affected in the N-terminal lipid-binding pocket. The specific PL transfer activity of the wild-type rPLTP preparations was between 0.3 and 0.4 nmol ng^{-1} h^{-1}, and the two mutant proteins displayed approximately 30% [30.6 ± 4.2% for L196W and 29.2 ± 8.7% for F464E (n = 4)] of the PL transfer activity as compared to wt rPLTP. To monitor HDL conversion by the rPLTP proteins, three different techniques were used: (i) The change in the HDL particle diameter was monitored by native GGE; (ii) the release of radioactively labeled apoA-I from the HDL was quantified by ultracentrifugation, and (iii) the electrophoretic mobility of the conversion products was analyzed using crossed immunoelectrophoresis.

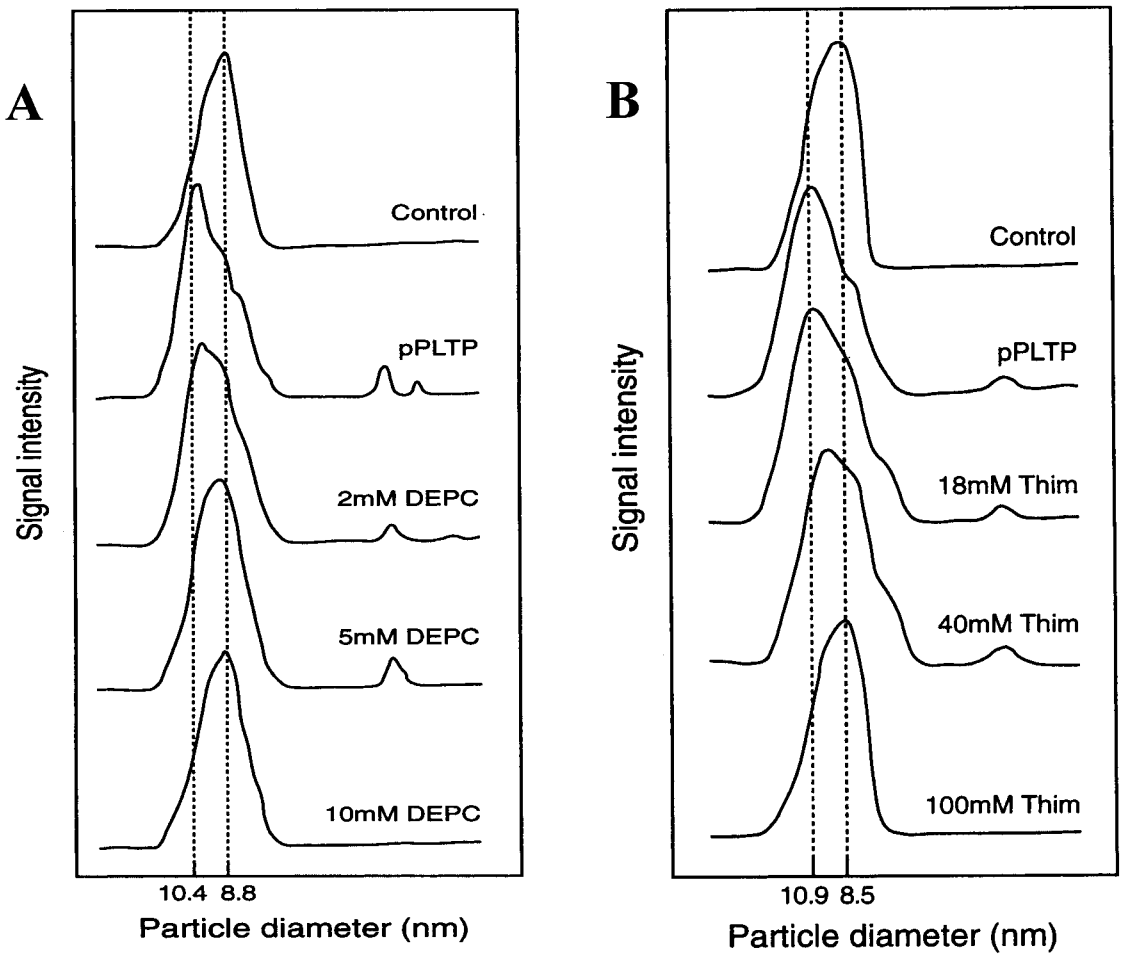

FIGURE 2: Effect of chemical modification on PLTP-mediated HDL conversion. Isolated plasma PLTP was incubated with increasing concentrations of diethylpyrocarbonate (A) or ethylmercurithiosalicylate (B) and used in the HDL conversion experiment as described in Experimental Procedures. After incubation for 24 h, an aliquot of the reaction mixture was resolved by native gradient gel electrophoresis, and the gel was stained with Coomassie Blue and scanned to determine the particle diameter (represented on the *x*-axis). Control is HDL_3 incubated in the absence of PLTP; pPLTP is HDL_3 incubated in the presence of purified plasma PLTP. The final inhibitor concentrations are indicated.

Incubation of HDL_3 in the presence of wt rPLTP induced formation of both large and small particles as revealed by GGE analysis (Figure 3). The major population of HDL particles shifted from the original size, diameter of 8.6 nm, to 11.1 nm particles during the 24 h incubation. A concomitant release of small lipid-poor apoA-I was observed. Similar results were obtained with isolated pPLTP included in each set of incubations as a control. The two PLTP mutants with reduced PL transfer activity were defective in their ability to induce HDL conversion. While wt rPLTP was able to convert most of the original HDL population into large and small particles in 24 h, there was still a substantial amount of the original particles remaining after incubation with the mutant proteins (Figure 3). In addition, the enlarged particles formed upon incubation with mutant rPLTP proteins were smaller compared with those formed by wt rPLTP. Importantly, we failed to detect formation of small apoA-I-containing particles upon incubation of HDL_3 with the mutant proteins. When the incubation was extended up to 48 h, no substantial difference in the GGE profile of the conversion products was observed compared to the 24 h time point (data not shown). These results demonstrate that compared to the wt PLTP, the mutant proteins induce only a modest size increment in HDL_3 particles and indicated that no release of small apoA-I particles takes place.

To quantitate the release of apoA-I from HDL, we used ^{35}S-labeled HDL_3 as a substrate for PLTP-mediated HDL conversion. The release of apoA-I by the wt rPLTP was both time- and concentration-dependent (Figure 4). As compared to samples incubated in the absence of added PLTP, 10 μg of wt rPLTP was able to release radioactively labeled apoA-I linearly throughout the incubation period, resulting in the release of 9% of the radioactivity at 48 h (Figure 4A). Furthermore, the level of apoA-I release increased with increasing concentrations of wt rPLTP (Figure 4B). The two mutant PLTP proteins, L196W and F464E, failed to release significant amounts of radioactively labeled apoA-I during the 24 h incubation (Figure 5). When the incubation period was extended to 48 h, the wt rPLTP-induced apoA-I release continued, while no release of the apoA-I by the mutant proteins was detectable (data not shown). These results demonstrate that the mutant proteins having a defect in their ability to transfer phospholipids are also incapable of releasing apoA-I during incubation with HDL_3.

It was recently shown that plasma of transgenic mice overexpressing human PLTP has increased capacity to generate preβ-mobile particles with apoA-I as a sole protein component (*12*). Analysis of the conversion products by crossed immunoelectrophoresis revealed that the apoA-I-containing particles released from the original α-mobile HDL

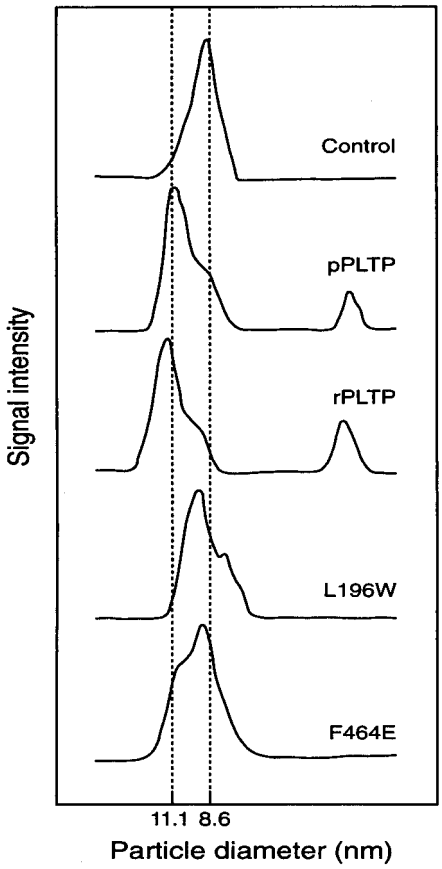

FIGURE 3: Change in particle diameter induced by wt and mutant rPLTP. Isolated recombinant PLTP was incubated with HDL_3 for 24 h. An aliquot of the reaction mixture was resolved by native gradient gel electrophoresis, and the gel was stained and analyzed with the Kodak software. The *x*-axis represents the particle diameter, and the *y*-axis shows the relative signal intensity. Control is HDL_3 in the absence of PLTP, pPLTP purified plasma PLTP, and rPLTP wild-type recombinant PLTP, and L196W and F464E are the mutant rPLTP proteins.

by wt rPLTP displayed preβ-mobility (Figure 6). Nanjee and co-workers have shown that the percentage of apoA-I in small HDL particles from human plasma is correlated with that in preβ-migrating species detected either by crossed immunoelectrophoresis or by ultracentrifugation (*29*). In agreement with this, we found that the quantity of preβ-HDL particles resolved by crossed immunoelectrophoresis was similar to the amount of released apoA-I determined by ultracentrifugation analysis (data not shown). The preβ-mobile particles could not be detected in HDL specimens incubated in the presence of the mutant rPLTP proteins (Figure 6). These results indicate that even though the mutant PLTP proteins are capable of inducing modest size increases in HDL particles, they are incapable of generating potentially antiatherogenic preβ-HDL.

DISCUSSION

Recent in vivo studies in animal models have demonstrated the importance of PLTP in HDL metabolism (*5*, *6*). Experiments in transgenic mice expressing human PLTP have demonstrated that the ability of plasma to generate lipid-poor apoA-I HDL with preβ-mobility correlates with plasma PL transfer activity (*12*). The data that were reported also demonstrated that the combination of increased plasma PLTP activity and improved preβ-HDL formation augments the ability of plasma to prevent cholesterol accumulation in macrophages. The authors suggest that PLTP is a potential antiatherogenic factor (*12*). On the other hand, the lack of PLTP in knockout mice leads to drastically reduced plasma phospholipid transfer activity (*6*), which results in impaired transfer of PL to nascent HDL and to severely reduced HDL levels in the plasma due to increased HDL catabolism (*30*). Thus, the two main activities of PLTP, the phospholipid transfer and HDL conversion, are physiologically important. To investigate the relationship between these two activities, we used in the present study chemically modified PLTP and recombinant PLTP proteins carrying point mutations in the N-terminal lipid-binding pocket.

Two chemical inhibitors, diethylpyrocarbonate and ethylmercurithiosalicylate, were used to modify PLTP. DEPC has been used to inhibit the enzymatic activity of proteins having histidine residues in their catalytic center, such as LCAT (*31*). Although no such center has been described for PLTP, its PL transfer function is affected by DEPC even at low concentrations (<0.5 mM). Thimerosal has previously been used to inhibit the PL transfer activity of PLTP (*19*, *27*). PLTP contains two free cysteine residues, at positions 22 and 335, and it is plausible that either one or both of these are covalently modified by Thimerosal. The results obtained with the modifying reagents indicate that gradual decrease in PL transfer activity is accompanied by a similar decrease in the level of PLTP-mediated HDL conversion. To further elucidate the relationship between the two activities of PLTP, we used two previously characterized PLTP point mutants. The mutants selected for this study have amino acid substitutions in the predicted N-terminal lipid-binding pocket of PLTP, resulting in proteins with severely reduced PL transfer activity (*14*). These substitutions do not seem to significantly affect the conformation of the protein as both are processed and secreted efficiently by transfected cells. Furthermore, insertion of a hexahistidine tag into the C-terminus of the proteins does not affect their secretion, specific phospholipid transfer activity, or HDL binding (data not shown). Thus, the mutant proteins comprise useful tools. In the conversion assays, these proteins exhibited severely reduced conversion activity. It is conceivable that if natural PLTP mutants with defects in their lipid-binding pockets occur in humans, the reverse cholesterol transport process in affected individuals will be compromised. This is implied by our present in vitro finding that the PLTP mutant proteins lack the property of releasing preβ-mobile apoA-I complexes despite their ability to induce a slight HDL size increment.

To study PLTP-mediated PL transfer and HDL conversion, Lusa et al. used reconstituted HDL particles containing either pyrene-labeled phospholipids in their surface lipid layer or pyrene-labeled cholesterol esters in the core (*15*). Incubations of labeled particles in the presence of PLTP demonstrated that the surface phospholipids were transferred rapidly and reached equilibrium prior to mixing of the cholesterol ester core, indicative of particle fusion. This study demonstrated differential kinetics for PL transfer and particle fusion but failed to show a causal relationship between the two processes. The authors presented a model which suggests that the initial reaction in HDL conversion is PL transfer. This leads to alteration in the surface pressure of HDL, release of apoA-I and phospholipids, and subsequent fusion of the unstable particles, resulting in the formation of large

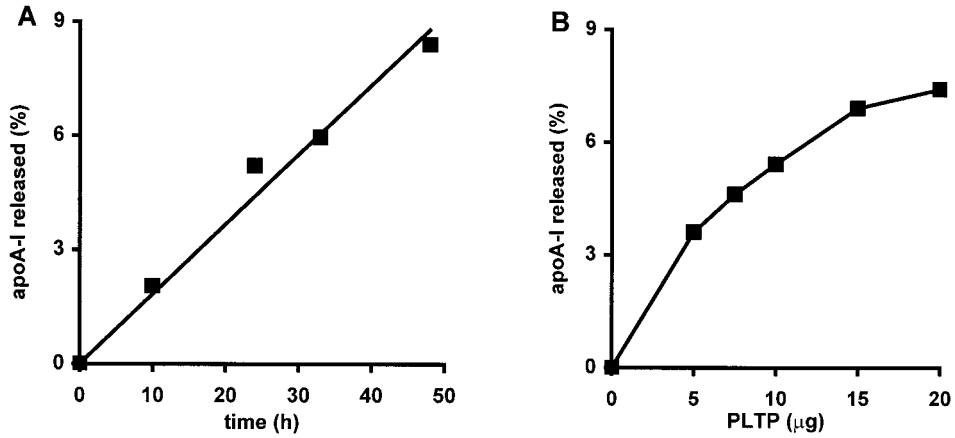

FIGURE 4: Release of radioactive apoA-I as a function of incubation time and PLTP concentration. Radioactively labeled HDL_3 was incubated with isolated rPLTP; the reaction mixture was centrifuged at a density of 1.21 g/mL, and the amount of radioactivity in the bottom fraction was determined as described in Experimental Procedures. For panel A (time dependence), 10 μg of rPLTP was incubated with HDL_3, and at the indicated time points, release of apoA-I was analyzed. For panel B (concentration dependence), HDL_3 was incubated with increasing amounts of rPLTP, and release of apoA-I was analyzed after incubation for 24 h. In both panels, the released apoA-I is presented as a percentage of the total amount of radioactivity. The background radioactivity (absolute value of 9%) representing the sample at 0 h (A) or HDL_3 incubated in the absence of rPLTP (B) has been subtracted.

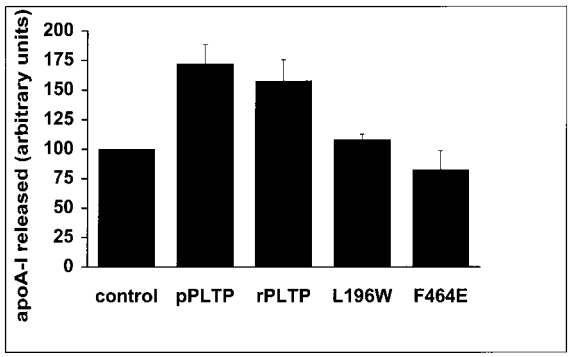

FIGURE 5: Release of radioactive apoA-I upon incubation of [^{35}S]-HDL_3 in the presence of wt or mutant rPLTP. The release of apoA-I was analyzed as described in the legend of Figure 4. The amount of apoA-I released after 24 h is expressed relative to the amount of HDL_3 incubated in the absence of PLTP (control, set at 100) and represents the mean ($\pm$ the standard error of the mean) of four independent experiments. pPLTP is purified plasma PLTP and rPLTP wild-type rPLTP, and L196W and F464E are the mutant rPLTP proteins.

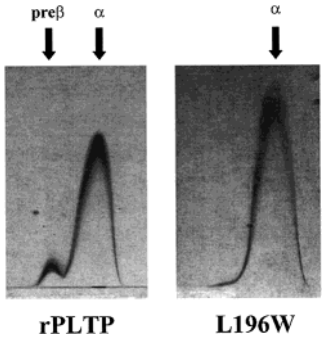

FIGURE 6: Crossed immunoelectrophoresis of HDL_3 after incubation with rPLTP. [^{35}S]HDL_3 was incubated in the presence of rPLTP for 24 h. The reaction mixture was analyzed by crossed immunoelectrophoresis as described in Experimental Procedures. Two representative HDL incubations, in the presence of wild-type rPLTP (in the left panel) and in the presence of mutant L196W (in the right panel) are shown. The electrophoretic mobility of the HDL particles in the first dimension is indicated on the top.

HDL. The fact that the PLTP-induced increase in HDL size is due to particle fusion and not aggregation was convincingly demonstrated by Korhonen et al. using a NMR technique (*16*). Our observation that there is a causal relationship between PLTP-mediated PL transfer and HDL conversion is in accordance with the model of Lusa et al.

Our results show that minor alterations in HDL particle size can be induced by PLTP having reduced PL transfer activity (caused either by chemical modification or by amino acid substitution). Theoretical calculations (*16*) suggest that fusion of two HDL_3 particles (diameter of 8.62 nm) results in a new particle with a diameter of 9.82 nm. Further fusion of three, four, and five HDL_3 particles leads to new particles with sizes of 10.66, 11.33, and 11.90 nm, respectively. The enlarged particles generated by the mutant proteins correspond in size to fusion products of two or three HDL particles. Thus, the mutant PLTP proteins are also able to start the fusion process, but fusion of a larger number of particles is hampered by the inefficient PL transfer. It is also possible that in a heterogeneous HDL particle population the mutant proteins are only able to fuse a specific subset of the particles. Desrumaux and co-workers have demonstrated that variations in the electrostatic charge of lipoproteins influence the PLTP-mediated phospholipid transfer process (*32*). Therefore, it is possible that the PL transfer takes place between specific subsets of HDL particles with an appropriate surface charge and thus causes net transfer of PL between given subpopulations leading to particle destabilization and conversion. Interestingly, a modest increase in particle size may also take place independently of apoA-I release. One can envision that after the initial particle fusion, continuation of the process causes an increase in particle surface pressure leading to the release of apoA-I.

To conclude, our results demonstrate that an efficient PLTP-mediated HDL conversion, characterized by both the increase in particle size and release of preβ-mobile HDL, is dependent on efficient phospholipid transfer. Only a small increase in HDL particle size can occur in the absence of apoA-I release. This suggests that even a low level of PL transfer is sufficient to start the fusion process between two or three HDL particles; however, the continuation of the process requires efficient PL transfer, and only then are antiatherogenic preβ-HDL particles released.

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