PLTP secreted by HepG2 cells resembles the high-activity PLTP form in human plasma

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Abstract **Plasma phospholipid transfer protein (PLTP) is an important regulator of plasma HDL levels and HDL particle distribution. PLTP is present in plasma in two forms, one with high and the other with low phospholipid transfer activity. We have used the human hepatoma cell line, HepG2, as a model to study PLTP secreted from hepatic cells. PLTP activity was secreted by the cells into serum-free culture medium as a function of time. However, modification of a previously established ELISA assay to include a denaturing sample pretreatment with the anionic detergent sodium dodecyl sulphate was required for the detection of the secreted PLTP protein. The HepG2 PLTP could be enriched by Heparin-Sepharose affinity chromatography and eluted in size-exclusion chromatography at a position corresponding to the size of 160 kDa. PLTP coeluted with apolipoprotein E (apoE) but not with apoB-100 or apoA-I. A portion of PLTP was retained by an anti-apoE immunoaffinity column together with apoE, suggesting an interaction between these two proteins. Furthermore, antibodies against apoE but not those against apoB-100 or apoA-I were capable of inhibiting PLTP activity. These results show that the HepG2-derived PLTP resembles in several aspects the high-activity form of PLTP found in human plasma.**—Siggins, S., M. Jauhiainen, V. M. Olkkonen, J. Tenhunen, and C. Ehnholm. **PLTP secreted by HepG2 cells resembles the high-activity PLTP form in human plasma.** ***J. Lipid Res.*** **2003.** 44: **1698–1704.**

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Both epidemiological and clinical studies provide strong evidence that low plasma HDL cholesterol concentration is a major risk factor for the development of coronary heart disease (CHD) (1–3). The ability of HDL to protect against the development of CHD has been well documented, and although the exact molecular mechanism(s) behind this finding is still unsolved, it is thought to be due to the role of HDL in the pathway of reverse cholesterol transport, i.e., the transport of cholesterol from peripheral cells to the liver for excretion (4). The HDL in human plasma consists of several subpopulations of particles of distinct structure, function, and composition. This heterogeneity, which is the result of continuous remodelling of HDL by plasma factors, has important implications in terms of the cardioprotective functions of HDL (5).

The regulatory role of phospholipid transfer protein (PLTP) in HDL metabolism is mediated via its two main functions, phospholipid transfer activity (6, 7) and the capability to modulate HDL size and composition in a process called HDL conversion (8–10). HDL conversion is fully dependent on the phospholipid transfer activity of PLTP (11). PLTP's role in the transfer of surface remnants from triglyceride-rich particles, VLDL, and chylomicrons to HDL during lipolysis is important for the maintenance of serum HDL levels (7, 12, 13). PLTP-facilitated fusion of HDL particles is accompanied by the release of poorly lipidated apolipoprotein A-I (apoA-I). This release of apoA-I in vivo results in the generation of preβ-HDL particles that are essential as cholesterol/phospholipid acceptors from cells in the reverse cholesterol transport process (4, 14). While the physiological function of PLTP in lipoprotein metabolism is far from resolved, the knowledge gained thus far on the role it plays in HDL remodelling and lipid transfer is substantial. These functions of PLTP are presumably antiatherogenic. However, studies employing PLTP knockout mice show that complete PLTP deficiency lowers atherosclerosis (15). On the other hand, transgenic mice overexpressing human PLTP display an increased risk for atherosclerosis due to lowered plasma HDL levels (16). Furthermore, with the discovery that PLTP exists in human plasma as two distinct forms, one with high activity and the other with low, it is conceivable that these forms have distinct functions in lipoprotein metabolism (17, 18). Therefore, the relative amount of each form of PLTP in plasma rather than total absence or overexpression will be a crucial determinant when evaluating PLTP's atherogenicity.

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Abbreviations: apoA-I, apolipoprotein A-I; HRP, horseradish peroxidase; IgG, immunoglobulin G.

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The association of PLTP with apolipoproteins in vitro (19) and in vivo (20) has been demonstrated. The low-activity form of PLTP in human plasma preferentially associates with apoA-I, and it is suggested that the high-activity form associates with apoE (18). Apolipoproteins may be essential for regulating PLTP activity and its ability to modify HDL in vivo. In addition, it has been suggested by Murdoch et al. that the immunoreactivity of the two forms of PLTP from plasma differ and that the use of monoclonal PLTP antibodies in ELISA mass assays may underestimate the mass of the active form (21). To overcome this discrepancy of antibody reactivity, to improve the mass determination of inactive and active PLTP, and to elucidate further the interaction of PLTP with apolipoproteins, we have used the human hepatoma cell line HepG2 as an in vitro model. We now report that PLTP secreted from HepG2 cells resembles in several aspects the high-activity PLTP form in human plasma: it is poorly immunodetectable in its native form, shows affinity for heparin, displays an apparent size of about 160 kDa, and cofractionates with apoE. Furthermore, we show that by pretreating the HepG2-derived PLTP with a strong denaturing anionic detergent, sodium dodecyl sulfate (SDS), we can significantly improve its immunochemical mass quantitation.

MATERIALS AND METHODS

Cell culture

Human hepatoblastoma-derived cells, HepG2, were obtained from the American Type Culture Collection (ATCC, reference HB-8065). Cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum, 100 U/ml penicillin/streptomycin, 1 mM sodium pyruvate, and 0.1 mM nonessential amino acids. The cells were cultured in 75 cm^2 flasks at 37°C under 5% CO_2 and 95% air. For the experiments, the cells were seeded into either 6 cm or 10 cm dishes and grown to 70% confluency in complete culture medium. The medium was then removed, and the monolayers were washed three times with phosphate-buffered saline (PBS) before the addition of serum-free DMEM. Incubation was performed for specific time intervals before the medium was collected and centrifuged at 2,000 rpm for 5 min to remove cells and cell debris. The medium was then stored on ice until used.

Antibodies

Polyclonal antibodies R164 (against the N terminus of PLTP, aa 18-144) and R176 (against the carboxyl terminus of PLTP, aa 425-493) as well as the monoclonal anti-PLTP antibody MAb JH59 were produced and characterized as previously described (22, 23). The polyclonal human anti-apoA-I antibody (R261) was produced in New Zealand White rabbits using highly purified apoA-I kindly provided by Dr. Peter Lerch, Swiss Red Cross, Bern, Switzerland. The polyclonal human apoB-100 antibody was raised in sheep using highly purified LDL as antigen. Commercial rabbit anti-human apoB-100 and rabbit anti-human apoE polyclonal antibodies were purchased from DAKO, Denmark. Monoclonal antibody 3D4 against apoA-I was a kind gift from Professor Yves Marcel, Montreal, Canada (24). The polyclonal human apoE antibody R107 was raised in rabbits with a standard immunization protocol using purified human plasma apoE as antigen.

PLTP activity assay

PLTP activity was measured using the radiometric assay described by Damen, Regts, and Scherphof (25) with minor modifications (8).

PLTP and apolipoprotein mass assays

Human PLTP mass was measured using a modification of the ELISA method of Huuskonen et al. (23). The ELISA was modified as follows: samples, standards, and controls were incubated with 0.1% SDS for 30 min at room temperature, and appropriate dilutions (final concentration of SDS 0.02%) were added to the wells. Highly purified human plasma active PLTP (23) was used as the primary standard. Further steps in the ELISA were the same as reported (23). An ELISA-based sandwich quantitation of human apoA-I was also performed. Briefly, the wells were coated with a polyclonal rabbit antibody, R34, against human apoA-I, and the bound protein was detected with a horseradish peroxidase (HRP)-conjugated rabbit anti-human apoA-I immunoglobulin G (IgG), R261. ApoE was also quantitated by ELISA using a polyclonal rabbit capture antibody, R107, to coat the wells and HRP-conjugated anti-human apoE polyclonal rabbit antibody (DAKO) for detection. In the apoB ELISA, the wells were coated with sheep anti-apoB and the antigen was detected with a rabbit anti-human apoB IgG-HRP conjugate.

Heparin-Sepharose affinity chromatography

Affinity chromatography was performed using a 5 ml HiTrap Heparin-Sepharose (H-S) column (Amersham Pharmacia Biotech, Uppsala). The column was equilibrated with 25 mM Tris-HCl buffer, pH 7.4, containing 1 mM EDTA, and 20 ml of the HepG2 serum-free culture medium was applied. The material that was bound to the matrix was eluted with 1 M NaCl at a flow rate of 1 ml/min, and 1 ml fractions were collected. The fractions were analyzed for PLTP activity and PLTP mass as well as apolipoprotein mass.

Size-exclusion chromatography

The H-S-bound fractions with high PLTP activity were combined and 1 ml was applied to a TSK 4000 size-exclusion (UltroPac Column TSK G4000SW, 7.5 × 600 mm, LKB Bromma, Sweden) silica column. The column was run in TBS buffer containing 0.2% Tween 20 at a flow rate of 0.25 ml/min, and 0.5 ml fractions were collected. The column was calibrated with standard proteins (BioRad Protein Standard No. 151-1901).

Inactivation of PLTP activity with antibodies

The H-S-bound fractions with high PLTP activity were combined and dialyzed against 10 mM Tris-HCl, 150 mM NaCl buffer, pH 7.4, containing 1 mM EDTA. The antibodies used were rabbit anti-PLTP (R176), anti-apoE (R107), anti-apoA-I (R34), sheep anti-apoB-100, and nonspecific rabbit IgG. All antibodies (15, 50, and 100 μg of IgG per incubation) were diluted in 10 mM Tris-HCl, 150 mM NaCl buffer, pH 7.4, containing 1 mM EDTA and added in equal volume to the H-S-bound PLTP. The samples (final volume 0.1 ml) were incubated overnight at 4°C, centrifuged, and assayed for PLTP activity.

Anti-apoE immunoaffinity chromatography

Polyclonal anti-apoE IgG (R107) and a control IgG were coupled to CNBr-activated Sepharose CL-4B according to the manufacturer's instructions (Amersham Pharmacia Biotech). The affinity columns contained 3.7 mg IgG/ml of the matrix. HepG2 culture medium was applied on the columns equilibrated with PBS, pH 7.4. Nonbound fractions were collected (fraction size, 4 ml) and the bound material was eluted with 0.1 M glycine, pH 2.8, into tubes containing 1 M Tris-HCl, pH 8.5, for neutralization.

General procedures

Proteins were resolved by SDS-PAGE according to the method of Laemmli (26), electrotransferred to Hybond-C membranes for Western blotting (27), and detected by enhanced chemiluminescence (ECL, Amersham) or conventional HRP color development reagent (BioRad). Nondenaturing polyacrylamide gradient 4–30% gel electrophoresis was performed using the method of Nichols, Krauss, and Musliner (28).

RESULTS

PLTP secretion by HepG2 cells

To study the secretion of PLTP from the liver, we used the human hepatoma cell line HepG2 as a model system. PLTP activity and concentrations of apolipoproteins A-I, B-100, and E were measured after 12 h, 24 h, and 48 h of cultivation in serum-free medium. A time-dependent secretion of phospholipid transfer activity into the medium could be observed. Between 12 h and 48 h, PLTP activity increased linearly to a maximum activity of 0.30–0.35 μmol/ml/h at 48 h (**Fig. 1**). Western blot analysis identified a single immunoreactive band of PLTP protein, the amount of which increased as a function of time. The molecular mass corresponded to ~80 kDa in SDS-PAGE (**Fig. 2**), a size similar to that of purified human plasma PLTP.

Because HepG2 cells have been reported to secrete a number of apolipoproteins (29–31), the secretion of apoA-I, apoE, and apoB-100 was analyzed by ELISA assays. The concentration of apoA-I, apoB-100, and apoE in the growth medium increased as a function of time, and at 48 h reached 8 μg/ml, 6.6 μg/ml, and 0.8 μg/ml, respectively (Figs. 1, 2). Western blot analysis of the growth medium demonstrated single protein bands for apoA-I, apoB-100, and apoE with the anticipated molecular masses (Fig. 2). The previously described ELISA assay for PLTP only detected trace amounts of the HepG2 PLTP protein in the culture supernatant, a result that was discrepant with the strong immunoreactivity observed upon Western blot analysis (Fig. 2). Murdoch and colleagues recently reported that the reactivity of different antibodies with the two PLTP forms varies

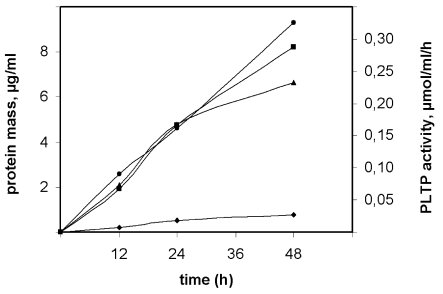

Fig. 1. Secretion of plasma phospholipid transfer protein (PLTP), apolipoprotein A-I (apoA-I), apoB-100, and apoE from HepG2 cells as a function of time. HepG2 cells were incubated in DMEM, 10% FBS, on 6 cm dishes until 70% confluent. The medium was then replaced with serum-free medium, and the cells were incubated up to 48 h. The culture supernatant was collected at the times indicated and analyzed for PLTP activity (circle), apoA-I (square), apoB (triangle), and apoE (diamond) concentration.

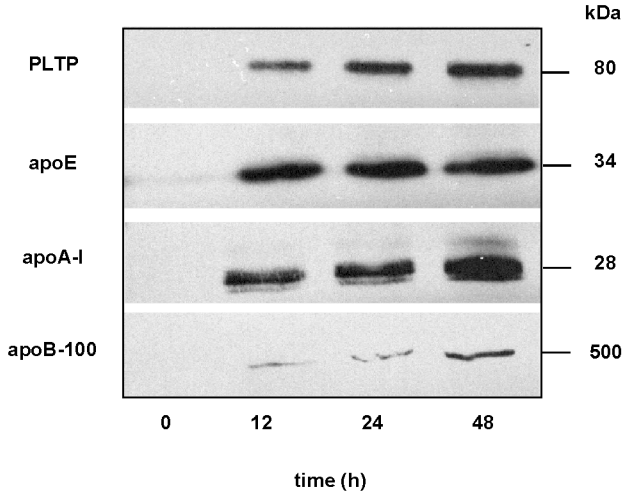

Fig. 2. Western blot analysis of PLTP and the apolipoproteins secreted from HepG2 cells. The culture supernatant from each time point (10 μl sample/well) was electrophoresed in 5% (apoB-100) or 12.5% (PLTP, apoE, and apoA-I) SDS-PAGE gels and then electrotransferred to Hybond-C membranes. Proteins were detected with commercial anti-apoB and anti-apoE antisera, and anti-PLTP (MAb JH59) or anti-apoA-I (MAb 3D4) antibodies, and the bound antibodies were visualized by enhanced chemiluminescence (ECL). Molecular masses of the analyzed proteins are displayed.

considerably (21). This implies that the epitope regions on PLTP may be differentially exposed in the two forms. Given the observation that the current ELISA for PLTP failed to detect mass for fractions containing abundant PLTP activity and protein (Figs. 1, 2), we considered the possibility that the HepG2-derived PLTP could be in a conformation poorly recognized by our antibodies and/or could participate in a complex in which epitopes are masked. We therefore subjected the HepG2 PLTP to a pretreatment with 0.1% SDS to unmask potentially hidden epitopes. To quantify the mass using the SDS-ELISA protocol, calibration was performed. For the standard, we used highly purified human plasma active PLTP (23). A standard curve was established after SDS pretreatment of the calibrator. A linear response was obtained in the concentration range of 1–100 ng/ml. This pretreatment step enabled us to reproducibly measure PLTP mass in the HepG2 cell supernatant (**Fig. 3**).

The PLTP mass values of the HepG2 culture supernatant that were determined by the SDS-ELISA assay increased as a function of time and correlated well with the activity measured. At 48 h, the specific PLTP activity (ratio of PLTP activity and mass) was 2.8–3.9 μmol/μg PLTP protein/h. The specific activity for the high-activity form in human plasma isolated by H-S affinity chromatography (18) and assayed by SDS-ELISA is 2.5–3.3 μmol/μg PLTP protein/h. As human plasma contains both high- and low-activity forms of PLTP (18, 32), it appears that the secreted HepG2 PLTP resembles the high-activity form.

Characterization of the secreted PLTP

To monitor the size of the nascent PLTP complexes secreted from HepG2 cells, we used nondenaturing gradient gel electrophoresis of HepG2 cell culture medium col-

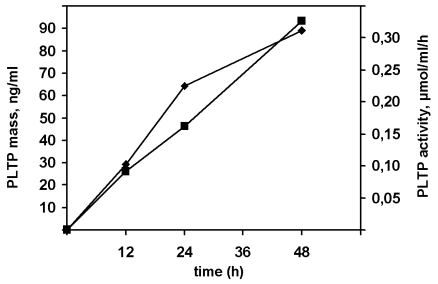

Fig. 3. PLTP mass secreted from HepG2 cells as a function of time. HepG2 cells were incubated in serum-free medium up to 48 h. The concentration of PLTP protein (diamond) secreted into the medium at the time points indicated was measured after pretreatment of the samples with sodium dodecyl sulfate (SDS). PLTP activity (square) was determined as described in the Materials and Methods section.

lected at 48 h. Western blot analysis of the gels revealed that PLTP migrated corresponding to the molecular mass range of 150–450 kDa (**Fig. 4**). This observation supports the notion that PLTP in the growth medium is not a monomer but part of a large complex.

PLTP in human plasma associates with apolipoproteins, forming different complexes of defined size, and has previously been shown to display affinity for heparin (18). Therefore, HepG2 cell culture medium collected at 48 h was subjected to H-S affinity chromatography (**Fig. 5**). Of the PLTP activity and mass applied, >90% was recovered as heparin-bound material that could be eluted with 1 M NaCl. Gradient elution (0–1 M NaCl) did not resolve two forms of PLTP, as both the activity and mass eluted together. Significant portions of apoB-100, apoE, and apoA-I coeluted with PLTP from the H-S column. The H-S elution fractions with PLTP activity and mass were combined and contained 0.8 µg/ml of PLTP, 1.2 µg/ml of apoE, 0.25 µg/ml of apoA-I, and a major portion of apoB-100.

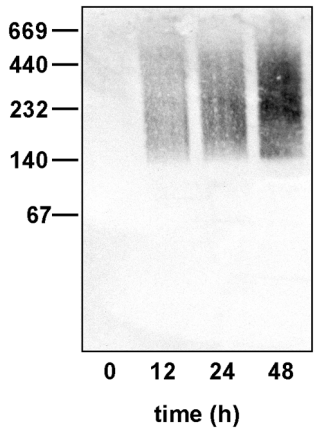

Fig. 4. Native gradient gel electrophoresis of PLTP secreted from HepG2 cells. HepG2 cells were incubated up to 48 h in 50 µl of the culture supernantant together with 40 µl of sample buffer, and were resolved on a 4–30% native gradient polyacrylamide gel. Proteins were electrotransferred to a Hybond-C membrane after presoaking the gel in 2.5% SDS-containing buffer. PLTP was detected with anti-PLTP MAb JH59, and the bound antibody was visualized by ECL. Migration positions of molecular mass markers are indicated.

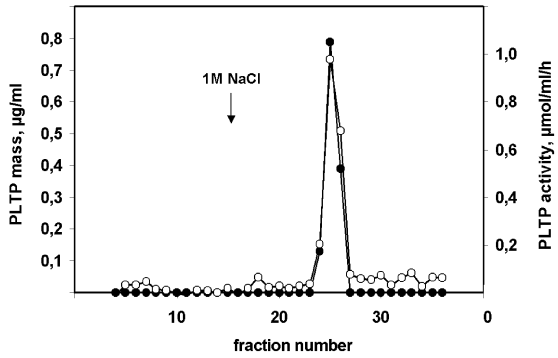

Fig. 5. Heparin-Sepharose (H-S) affinity chromatography of HepG2 cell culture medium. HepG2 cells were incubated in serum-free medium for 48 h, and a total volume of 20 ml was applied to a 5 ml HiTrap H-S column. The column was washed with 25 mM Tris-HCl buffer, pH 7.4, containing 1 mM EDTA to remove nonbound material. Heparin-bound material was then eluted with 1 M NaCl. Fractions were analyzed for PLTP activity (open circle) and mass (closed circle).

To further characterize the size and composition of the PLTP-containing complex in the HepG2 cell growth medium, size-exclusion chromatography and Western blot analysis were used. H-S elution fractions exhibiting high PLTP activity (1.0–1.5 µmol/ml/h) were combined, dialyzed against TBS, adjusted to 0.2% Tween 20 and applied to a TSK 4000 column. Detergent was used in this chromatography step to avoid nonspecific adsorption of proteins to the matrix. In the absence of detergent, PLTP activity was retarded by the matrix (data not shown). A major portion (80%) of the PLTP activity and all of the detectable PLTP mass applied were recovered in a position corresponding to a molecular mass of about 160 kDa (**Fig. 6**). To assess the particle composition of PLTP eluting at this 160 kDa position, the apolipoprotein constituents in the elution fractions were analyzed with ELISA and Western blotting. ApoA-I and apoB-100 did not coelute with PLTP, as the fractions containing apoA-I were located within the 44 kDa region, corresponding to poorly lipidated apoA-I (33), and fractions containing apoB-100 were within the size region of 670 kDa (data not shown). However, at the 160 kDa position, PLTP did coelute with apoE. Thus, the elution patterns of PLTP and apoE were similar during both chromatography steps, i.e., H-S and size-exclusion chromatography. Fractions in the 160 kDa region (fractions 42–44 were combined) contained 71 ng/ml PLTP and 128 ng/ml apoE, corresponding to an approximate molar ratio of 1:4.

Inhibition of PLTP activity with antibodies

To study whether active PLTP is associated with apoE, coeluting with it in the H-S affinity and size-exclusion chromatography steps, we carried out immuno-inhibition experiments using antibodies specific for PLTP, apoE, apoA-I, and apoB-100. The experiments were performed using the PLTP preparation that eluted from the H-S column and contained apoA-I, apoB-100, and apoE. Incubation of the preparation with the PLTP antibody resulted in an antibody concentration-dependent inhibition of PLTP

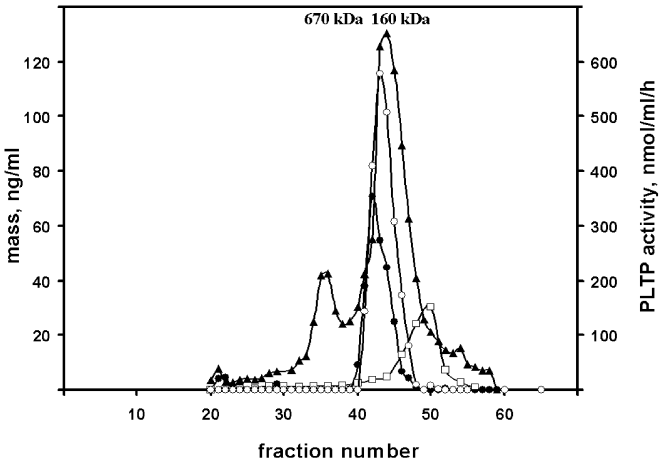

Fig. 6. Size-exclusion chromatography of the H-S-bound proteins. The H-S-bound fractions with high PLTP activity were combined, dialyzed against TBS buffer, and adjusted to 0.2% Tween 20, and 1 ml was applied to a TSK 4000 size-exclusion column. The material was eluted in TBS buffer containing 0.2% Tween 20. PLTP activity (open circle) and PLTP mass (closed circle), apoE mass (triangle), and apoA-I mass (square) were measured. The elution positions for 670 kDa and 160 kDa are indicated.

activity that reached 87% at an IgG concentration of 0.5 mg/ml (**Fig. 7**). Antibodies against apoA-I or apoB-100 did not significantly inhibit PLTP activity, but antibodies against apoE had a marked inactivating effect on PLTP, 83% inhibition at an IgG concentration of 1 mg/ml.

Anti-apoE immunoaffinity chromatography of HepG2 cell culture medium

To further study the interaction between PLTP and apoE, we subjected HepG2 cell culture medium to chromatography on an anti-apoE affinity column. In addition to apoE, which was quantitatively bound to the column, a portion of PLTP applied was bound and could be eluted together with apoE at low pH, as demonstrated by Western blot analysis (**Fig. 8**). Neither of the two proteins was significantly retained by a control IgG column (data not shown). These data, combined with the antibody inhibition results, indicate that HepG2 PLTP and apoE form a complex.

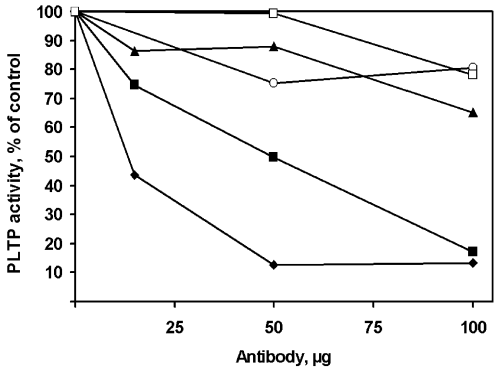

Fig. 7. Immuno-inhibition of PLTP activity. HepG2 PLTP collected at the 48 h time point and enriched by H-S affinity chromatography was incubated overnight at 4°C with increasing concentrations of antibodies against PLTP (closed diamond), apoA-I (closed triangle), apoB-100 (open circle), apoE (closed square), or nonspecific immunoglobulin G (open square). The PLTP activity in the samples was thereafter measured. The data shown represent two independent experiments, each carried out in duplicate. The results are represented as the percent activity remaining after the antibody incubations. The activity of the sample incubated with buffer only was set at 100% (=170 nmol/ml/h).

DISCUSSION

Human plasma contains two distinct forms of PLTP, one with high and the other with low phospholipid transfer activity (17, 18, 21). How these two forms are generated is, however, unknown. It has been suggested that PLTP is secreted as a high-activity form that is then converted into the low-activity form during a process that occurs in the circulation, possibly in connection with the transfer of lipolytic surface remnants of triglyceride-rich lipoproteins to HDL (18). Considering the PLTP mRNA expression pattern and the relative size differences of the PLTP-expressing human tissues, the liver is probably the most important source of circulating PLTP (34, 35). In the present study, we used the human hepatoma cell line HepG2 as a model for studying PLTP secretion from liver cells. The HepG2 cell system is a relevant model with regard to human hepatic fatty acid uptake, lipid synthesis, apolipoprotein synthesis, and lipolytic enzyme and transfer protein synthesis (LCAT, hepatic lipase, cholesteryl ester transfer protein), as well as apoB-100-lipoprotein assembly and secretion (31, 36). While this study supports the previous findings that PLTP activity accumulates in the culture medium of HepG2 cells (37, 38), no detailed characterization of the secreted PLTP has been carried out before.

Even though the HepG2 cells secreted significant amounts of active PLTP into the culture supernatant, our sandwich ELISA for PLTP protein quantitation (23) detected hardly any PLTP protein. However, when analyzed by SDS-PAGE and Western blotting, an appreciable amount of PLTP protein was observed. This discrepancy in protein

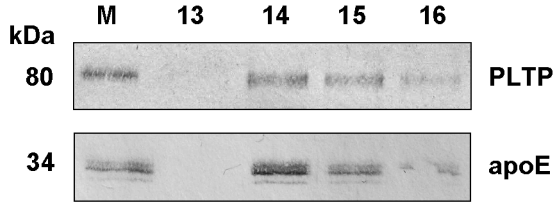

Fig. 8. Anti-apoE immunoaffinity chromatography of HepG2 cell culture medium. HepG2 cell culture medium (5 ml) collected at the 48 h time point was applied onto an anti-apoE affinity chromatography column and the chromatography performed as described in Materials and Methods. The retained proteins eluted with low pH were analyzed for apoE and PLTP by Western blot analysis using anti-PLTP (MAb JH59) or anti-apoE (R 107) antibodies and horseradish peroxidase detection. Molecular masses of the analyzed proteins are indicated. Identification of the lanes: M, HepG2 cell culture medium; 13–16, anti-apoE column elution fractions.

detection between the two assays can only be explained by a marked difference in the ability of the plasma high- and low-activity PLTP forms to react with specific antibodies (21). The ELISA methods used in studies on PLTP distribution in human plasma have revealed low amounts of PLTP protein in the fractions displaying the highest phospholipid transfer activity (17, 18, 32). These results may be due to dissimilar reactivity of antibodies for epitopes differentially exposed on the surface of PLTP in the high- and low-activity forms of the protein. SDS is a strong anionic denaturing detergent used in several applications to improve immunochemical detection of proteins (39, 40). In order to reveal hidden epitopes by denaturation, we decided to treat the HepG2 culture supernatants with 0.1% SDS prior to ELISA analysis. Upon SDS pretreatment, PLTP immunoreactivity from the HepG2 culture supernatants increased, thus suggesting that the PLTP secreted by HepG2 cells may resemble, in its immunological properties, the high-activity form of plasma PLTP. Therefore, in its native form, the HepG2 PLTP displays a conformation and/or participates in protein-protein or protein-lipid interaction(s) that renders the epitope(s) essential for recognition inaccessible. The low reactivity shown here, as well as the high specific activity of the HepG2 PLTP calculated based on the SDS-ELISA results, implies that there is no inactive PLTP secreted. Furthermore, we have been unable to obtain any other evidence that would suggest that HepG2 cells secrete PLTP as a low-activity complex reminiscent of that found in human plasma.

As with plasma PLTP (18), it was possible to enrich the HepG2 PLTP by H-S affinity chromatography, indicating that PLTP itself shows affinity for heparin, as earlier suggested (41), or binds to heparin indirectly through an interaction with another heparin binding protein, such as apoE (42). To investigate this potential apolipoprotein-PLTP association, we analyzed the PLTP by nondenaturing gradient gel electrophoresis after H-S enrichment and from the HepG2 culture supernatant. We were able to show that the secreted PLTP is not a monomer but, rather, a part of a larger complex. Furthermore, in size-exclusion chromatography, a majority of the HepG2 PLTP eluted at the position corresponding to the size of ~160 kDa. This size is highly similar to that determined for the high-activity form of plasma PLTP (18, 21, 43). Upon H-S affinity chromatography, HepG2 PLTP coeluted with apoB-100, apoE, and apoA-I. However, during separation by size-exclusion chromatography, PLTP was separated from apoB-100 and apoA-I while retaining full activity, but yet coeluted with apoE. This finding is in agreement with the previous observations that the high-activity form of plasma PLTP copurifies with apoE (18) and that part of plasma PLTP migrates with apoE upon two-dimensional native gradient gel electrophoresis (20). Therefore, the data here do not support the interpretation of Murdoch et al. that the active form of PLTP is associated with apoA-I-containing particles and the inactive form with apoE-containing particles (21).

The possibility that the HepG2 PLTP may functionally associate with apoE was further studied by immunological approaches. Antibodies against apoE were found to abolish the phospholipid transfer activity of HepG2 PLTP, while antibodies against apoA-I and apoB-100 had no significant effect. Furthermore, a portion of PLTP was bound together with apoE to an anti-apoE affinity column, demonstrating an interaction between these two proteins.

Even though the present data imply that there is functional interplay between PLTP and apoE secreted by HepG2 cells, mice lacking the apoE gene display normal plasma PLTP activity (M. Jauhiainen and K. Aalto-Setälä, unpublished observations). Thus, at least in mice, apoE is not crucial for PLTP activity and other apolipoproteins seem to be able to substitute for apoE as partners of PLTP. The PLTP secreted by HepG2 cells resembles the high-activity form of PLTP circulating in plasma (18) in its immunoreactivity, affinity for heparin, apparent size, and its cofractionation with apoE. Therefore, our results provide support for the hypothesis that PLTP enters the circulation in an active form that is later converted into the inactive complex (17, 18). The HepG2 cell model now provides us with a tool for studying the mechanisms by which active PLTP is converted to the low-activity form associated with apoA-I in plasma.

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