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Biochemical and Biophysical Research Communications 331 (2005) 333–340
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Apolipoprotein E activates the low-activity form of human phospholipid transfer protein ☆

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Received 9 March 2005
Available online 2 April 2005

Abstract

Phospholipid transfer protein (PLTP) exists in a high-activity (HA-PLTP) and a low-activity form (LA-PLTP) in the circulation. LA-PLTP is associated with apoA-I while the HA-PLTP complex is enriched with apoE. To study the interaction of PLTP with apolipoproteins, we carried out surface plasmon resonance analyses. These demonstrated a concentration-dependent binding of recombinant human PLTP, which represents an active PLTP form, and LA-PLTP to apoE, apoA-I, and apoA-IV within a nanomolar K_D range. To study whether LA-PLTP can be transformed into an active form, we incubated it in the presence of proteoliposomes containing apoE, apoA-I or apoA-IV. The apoE proteoliposomes induced a concentration-dependent activation of LA-PLTP. ApoA-IV proteoliposomes also activated LA-PLTP in a concentration-dependent manner, whereas apoA-I proteoliposomes had no such effect. These observations suggest that PLTP is capable of interacting with apoE, apoA-I, and apoA-IV, and that these interactions regulate PLTP-activity levels in plasma.

Keywords: Apolipoproteins; Activation; Lipid transfer proteins; Phospholipid transfer protein; HA-PLTP; LA-PLTP

Numerous clinical and epidemiological studies have demonstrated the inverse correlation between high density lipoprotein (HDL) cholesterol and the risk of coronary heart disease [1,2]. This is often explained by the ability of HDL to remove excess cholesterol from peripheral (non-hepatic) cells into the plasma compartment and to transport it to the liver for excretion. This process is known as reverse cholesterol transport [3].

HDL metabolism is under the control of multiple proteins [4]. These include phospholipid transfer protein (PLTP), lecithin:cholesterol acyltransferase (LCAT), and cholesteryl ester transfer protein (CETP). Plasma PLTP has an essential role in the transfer of surface remnants from triglyceride-rich particles, very low density lipoproteins (VLDL), and chylomicrons (CM), to HDL during lipolysis. This is of importance for the maintenance of plasma HDL levels [5–7]. PLTP also modulates the size and composition of HDL particles [8,9]. Current observations on the presence of PLTP in macrophages and atherosclerotic lesions suggest that PLTP could function either as an anti-atherogenic molecule by facilitating cholesterol efflux or as a

☆ *Abbreviations:* PLTP, phospholipid transfer protein; LA-PLTP, low-activity form of PLTP; apo, apolipoprotein; PC, phosphatidylcholine; PL, phospholipids; UC, unesterified cholesterol.

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0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.03.164

pro-atherogenic molecule by mediating lipid retention [10,11]. PLTP may also be a key factor in the reverse cholesterol transport process since it was shown to interact with ATP-binding cassette transporter A1 (ABCA1) on macrophages and facilitate cholesterol and phospholipid efflux [12].

The development of a PLTP mass determination assay revealed the presence of two forms of PLTP in plasma, one with high activity (HA-PLTP) and the other with low activity (LA-PLTP). The two forms are associated with macromolecular complexes of different size and composition when isolated from plasma [13,14]. HA-PLTP co-purifies with apolipoprotein E (apoE), and active PLTP secreted by HepG2 human hepatoma cells is also associated with apoE [15], whereas LA-PLTP is associated with apoA-I [14]. These observations are in line with our working hypothesis in which PLTP is secreted as a high-activity complex, and during phospholipid (PL) transfer of surface remnants from triglyceride-rich lipoproteins to HDL, it becomes complexed with apoA-I and loses measurable PL transfer activity.

Considering the significant roles of apolipoproteins in the activation of LCAT and CETP [16–19], we studied the interaction of PLTP with apolipoproteins and their ability to transform LA-PLTP into HA-PLTP.

Materials and methods

Reagents. Unesterified cholesterol (UC), egg-yolk phosphatidylcholine (egg PC), and dimyristoyl phosphatidylcholine (DMPC) were obtained from Sigma–Aldrich (St. Louis, MO). Thrombin protease was purchased from Amersham Pharmacia (Uppsala, Sweden).

Isolation and purification of PLTP. Recombinant PLTP (r-PLTP) was produced using a baculovirus/insect cell system and purified as described previously [20–22]. Two chromatographic steps, hydroxylapatite chromatography (Bio-Gel HTP, Bio-Rad Laboratories, Hercules, CA) and Ni–NTA Superflow Agarose (Qiagen, Valencia, CA) were used to purify r-PLTP [22].

LA-PLTP was isolated as described [14]. In addition to PLTP, the complex contained apoA-I, phospholipids (PL; mainly phosphatidylcholine, PC), and unesterified cholesterol (UC). The molar ratios for PLTP:apoA-I:PL:UC were 1:2:26:9, respectively.

SDS–PAGE and Western blotting revealed that the size of r-PLTP (75 kDa) was slightly smaller than the size of LA-PLTP purified from human plasma (80 kDa) due to differences in the degree of glycosylation [21].

Assays for PLTP activity and concentration. PLTP activity was measured using the radiometric assay described by Damen et al. [23] with minor modifications [8]. PLTP mass was assayed with an ELISA method [22].

Preparation of apolipoproteins. ApoE constructs were obtained from Dr. Karl H. Weisgraber (Gladstone Institute of Cardiovascular Disease, San Francisco, CA). The cDNAs for human apolipoprotein E_2, E_3 or E_4 were cloned into a modified pET32a(+) vector (Novagen, EMD Biosciences, Darmstadt, Germany), which encodes an N-terminal His_6 tag and a fusion partner, thioredoxin [24].

Recombinant apoE was produced in Escherichia coli BL21 (DE3) and purified using His-Bind Resin Chromatography Kit (Novagen) as described [24]. Thrombin digestion was performed at room temperature for 30 min at a ratio of 1:500 (thrombin:apoE, w/w). Delipidation of apoE was carried out as described [25]. Heparin–Sepharose affinity chromatography (HiTrap Heparin, Amersham Biosciences, Uppsala, Sweden) was used as the final step in apoE purification. Briefly, the delipidated apoE was diluted in PBS containing 5 M urea and 0.1% of 2-mercaptoethanol, pH 7.4. The apoE sample was then loaded onto a 5 ml heparin–Sepharose column equilibrated with the 5 M urea buffer at a flow rate of 1 ml/min. The bound apoE was eluted from the column with 1 M NaCl in 5 M urea buffer and dialyzed against 100 mM NH_4HCO_3, pH 8.0. Purified apoE isoforms displayed one band staining in SDS–PAGE analysis with an apparent molecular mass of 34 kDa (data not shown).

Plasmid DNA of pET14b/WT-apoA-IV containing a His_6 tag was obtained from Dr. Philippa Talmud (Royal Free and University College London Medical School, London, UK) and transformed into *E. coli* BL21 (DE3) cells. The cell suspension was thawed on ice, diluted in PBS, and lysed using lysozyme (100 μg/ml). After adding the protease inhibitors (Complete, EDTA-free, Roche Diagnostics GmbH, Mannheim, Germany), the mixture was incubated on ice for 15 min. The cells were disrupted by sonication and freeze–thaw cycles. After centrifugation, the supernatant was loaded on a His-Bind chromatography column using a His-Bind Resin Chromatography Kit (Novagen). Purified apoA-IV displayed one band staining in SDS–PAGE analysis with a molecular mass of 46 kDa (data not shown).

Highly purified apoA-I was kindly provided by Dr. Peter Lerch (Swiss Red Cross, Bern, Switzerland) [26].

Surface plasmon resonance analysis. Binding of r-PLTP and LA-PLTP to apoE, apoA-I or apoA-IV was studied with surface plasmon resonance (SPR) analysis in a Biacore 2000 biosensor (Biacore AB, Uppsala, Sweden). Interaction of an analyte with an immobilized ligand on the sensor chip results in changes in SPR signals that are recorded in real time as resonance units (RU) [27,28]. For each experiment, the flow cells of a CM5 biosensor chip were prepared by covalently attaching saturating amounts of apoE, apoA-I or apoA-IV via standard amine coupling according to the manufacturer's instructions (Biacore AB, Uppsala, Sweden). The coupling densities were 5400 RU for $apoE_2$, 4200 RU for $apoE_3$, 5500 RU for $apoE_4$, 4500 RU for apoA-I, and 8000 RU for apoA-IV. A wide range of concentrations of r-PLTP (0.25–220 nM) and LA-PLTP (0.75–190 nM) diluted in PBS were used as analytes. Recombinant PLTP, which represents an active PLTP form, was primarily chosen to be used as an analyte to minimize the effects of contaminating proteins on binding. The binding properties of r-PLTP were compared to those of LA-PLTP. The binding of r-PLTP and LA-PLTP to immobilized apoE isoforms was assayed at +25 or +37 °C for 5–10 minutes at a flow rate of 10–30 μl/min. Interactions of both PLTP forms with apoA-I and apoA-IV were analyzed at a lower temperature, +15 °C, to delay fast dissociation of the analyte. As a negative control, an empty flow cell was used. Sensor chip surfaces were initially regenerated at pH 2.0 with a 30 s hydrochloric acid pulse and after each injection by a 1 h wash with running buffer (PBS).

The data were evaluated by first subtracting the sensorgram of the control flow cell from the sensorgrams of flow cells containing apoE, apoA-I or apoA-IV. The binding kinetics was fitted to the simultaneous k_a/k_d determination feature of the Biaevaluation 3.1 software package employing the natural logarithmic Langmuir binding (1:1) model to which the two state model and other multicomponent models were compared.

Preparation and characterization of proteoliposomes. To examine the roles of apolipoproteins in the regulation of PLTP activity, proteoliposomes containing either apoE, apoA-I or apoA-IV as protein component, egg PC, and UC were prepared by the cholate dialysis technique [29,30]. Briefly, PC in chloroform and UC in ethanol were mixed, dried under nitrogen, and kept in a vacuum to remove traces of solvents. The dry residue was resuspended in 1 ml of 10 mM Tris–HCl, 150 mM NaCl, and 1 mM EDTA, pH 7.4 (TNE buffer). Apolipoprotein (1–5 mg) was added to the mixture to give a PC:UC:apolipopro-

Table 1
Characterization of the proteoliposomes presented as molar ratios of protein, phospholipid (PL), and unesterified cholesterol (UC)

Proteoliposomes	Protein	PL	UC	Diameter (nm)[a]
$ApoE_2$	1	68	15	9.3, 9.5, 13.5
$ApoE_3$	1	67	13	9.5, 11.6, 13.0
$ApoE_4$	1	104	18	9.3, 12.3, 13.2
ApoA-I	1	57	13	8.0, 8.2, 10.4, 11.2
ApoA-IV	1	125	19	7.9, 10.4, 11.4, 12.0

[a] The diameter of the proteoliposomes as determined by native gradient gel electrophoresis. The sizes of the main populations have been underlined. The molar ratios of protein, PL, and UC represent those determined for the entire proteoliposome population.

tein molar ratio of 70:12.5:1. Sodium cholate (0.725 M, Sigma–Aldrich, St. Louis, MO) was added to the lipid-apolipoprotein suspension, in which the final PC to sodium cholate molar ratio was approximately 1:1. The suspension was incubated at +22 °C for 20 min after which it was dialyzed against TNE buffer (3 × 5 L) to remove excess cholate. Liposomes containing no protein were prepared by sonication using the same lipid molar ratios.

The lipid and protein composition of the proteoliposomes, as well as the particle size, is presented in Table 1. Particle size of the proteoliposomes was analyzed by size-exclusion chromatography on a Superose 6 HR (10/30) and non-denaturing PAGE [31] using self-made 4–30% gradient gels.

Preparation of spherical reconstituted HDL particles. To ensure that the components of native HDL_3 particles, isolated from human plasma, do not have an influence on the activation of LA-PLTP by the proteoliposomes, we prepared spherical reconstituted HDL particles (r-HDL) containing apoA-I as the only apolipoprotein [32] and compared the performance of these particles as phospholipid acceptors to that of native HDL_3 in our radiometric PLTP-activity assay.

Activation experiments. In each set of experiments 100 ng of LA-PLTP, as measured by ELISA [22], was incubated with apoE-, apoA-I- or apoA-IV-proteoliposomes in a total volume of 0.5 ml TNE buffer. LA-PLTP:apolipoprotein molar ratios varied from 1:250 up to 1:2000. Preincubations were carried out at +37 °C for 30 min. The preincubated samples, 50 ng LA-PLTP, in duplicate, were thereafter assayed for PLTP activity at +37 °C for 45 min. In each set of experiments, liposomes or proteoliposomes without LA-PLTP were used as controls. LA-PLTP alone in TNE-buffer was used as an additional control.

Other methods. Lipoproteins were isolated from human plasma by sequential ultracentrifugation using solid KBr to adjust densities [33]. Total cholesterol (Kit 1489232, Roche Diagnostics GmbH, Mannheim, Germany) and phospholipids (Kit 999-54006, Wako Chemicals GmbH, Neuss, Germany) were measured using commercial kits.

Protein concentration was determined by the method of Lowry et al. [34] using bovine serum albumin as a standard. SDS–PAGE was carried out by the method of Laemmli [35] and Western blotting as described by Towbin et al. [36].

Results

Binding of r-PLTP and LA-PLTP to apoE, apoA-I, and apoA-IV

The binding of r-PLTP and LA-PLTP to apoE, apoA-I, and apoA-IV was characterized using surface plasmon resonance analysis on a Biacore 2000 instrument. Recombinant proteins, PLTP, apoE, and apoA-IV, or highly purified apoA-I, were used to minimize the effects of contaminating serum proteins on the interactions. The sensorgrams clearly demonstrate a concentration-dependent binding of PLTP to apoE and apoA-I within a nanomolar range for both r-PLTP and LA-PLTP, as illustrated in Fig. 1. Both forms of PLTP also showed binding to apoA-IV. Presence of PLTP on the flow cell surface was demonstrated by using a polyclonal anti-PLTP antibody (data not shown). This provided evidence that a stable association of proteins and not only a donation of PL was taking place. The data presented in Fig. 1 were used to evaluate the effect of each PLTP concentration injected on the binding response at a steady-state phase of each interaction. These equilibrium-binding responses are presented in Fig. 2, which demonstrates that in the cases where saturation was reached, the estimated K_D values were comparable with the K_D values derived from the reaction rates (Table 2).

The binding affinities of the two forms of PLTP to apoE, apoA-I, and apoA-IV were determined from rate equations by simultaneous k_a/k_d simulated kinetics. The kinetic fitting data obtained using a Langmuir model are shown in Table 2. The reliability of the results is illustrated by the average χ^2 values of 2.65 (r-PLTP) and 0.50 (LA-PLTP). It was of interest to note that the binding of both forms of PLTP to the isoforms of apoE: $apoE_2$, $apoE_3$, and $apoE_4$, is different. $ApoE_2$ was the most stable binder due to the slow dissociation rates (k_d) of the analytes, whereas binding of both forms of PLTP to the other apolipoproteins was driven by fast association kinetics. The K_D values and interaction rates of r-PLTP were similar to those of LA-PLTP with apoE. However, LA-PLTP associated with apoA-I 10 times faster than r-PLTP. Since LA-PLTP also dissociated rapidly from apoA-I, the corresponding K_D value was relatively high. In contrast to apoA-I, the affinity of apoA-IV was higher for LA-PLTP than for r-PLTP. This was due to a fast dissociation rate (k_d) of r-PLTP from apoA-IV. From these experiments we conclude that the two forms of PLTP interact with apoE, apoA-I and apoA-IV, albeit with different affinities and interaction rates.

Activation of the low-activity form of PLTP

To examine the possible physiological role of LA-PLTP binding to apolipoproteins, we studied whether LA-PLTP could be activated by apoE, apoA-I, or apoA-IV. For this purpose, we incubated proteoliposomes for 30 min at +37 °C with LA-PLTP, which had no detectable PL transfer activity, at different molar ratios as described under Materials and methods. The incubation with apoE proteoliposomes resulted in a concentration-dependent activation of LA-PLTP. Similar activation was obtained using proteoliposomes prepared

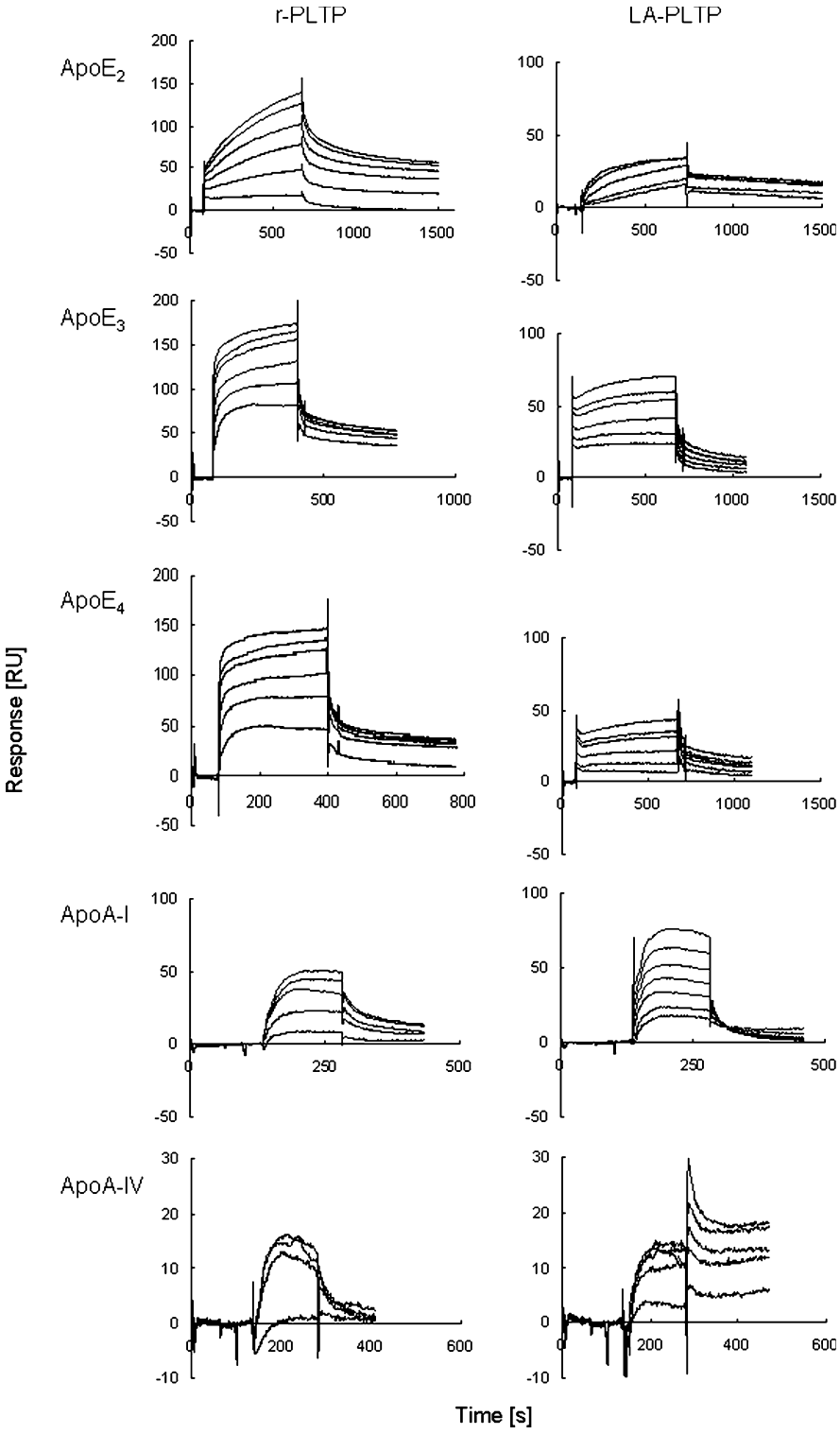

Fig. 1. Surface plasmon resonance analysis of the binding of r-PLTP (the left-hand column) and LA-PLTP (the right-hand column) to apoE, apoA-I, and apoA-IV immobilized on CM5 sensor chips. Representative sensorgrams of PLTP-binding responses are presented. The binding of both forms of PLTP was analyzed using a concentration range of 10–180 nM.

from the different apoE isoforms: apoE_2, E_3 or E_4. The results from these experiments are summarized in Fig. 3. After incubation with the highest concentration of apoE proteoliposomes, a mean PLTP activity of 264 nmol/ml/h was reached. We did not observe a significant difference in LA-PLTP activation whether native HDL_3 or spherical r-HDL particles were used as acceptors in the PLTP-activity assay (data not shown).

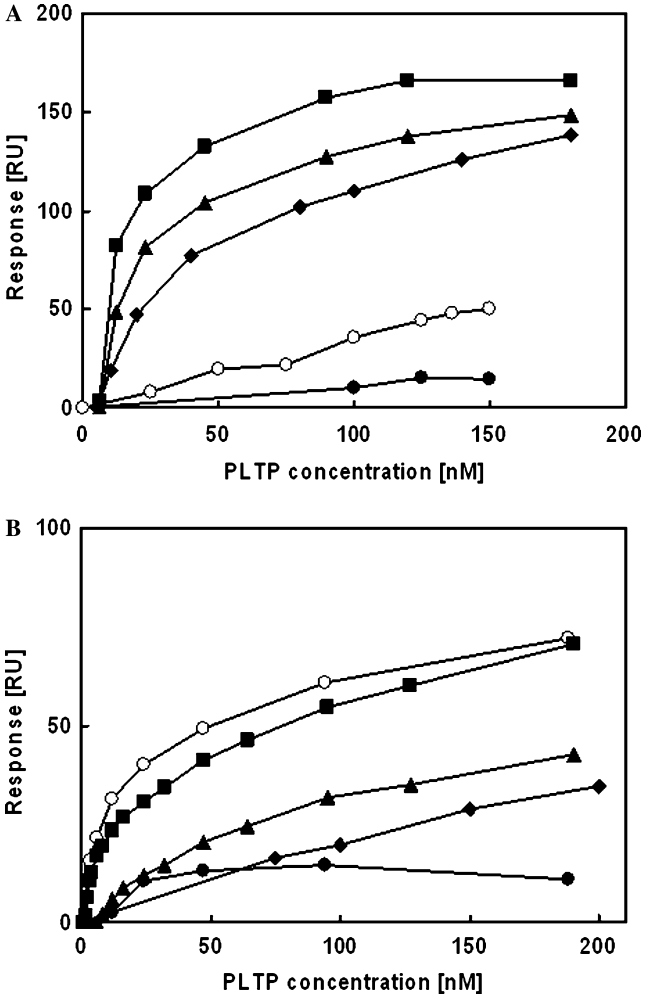

Fig. 2. Effect of r-PLTP and LA-PLTP concentration on binding to apoE, apoA-I, and apoA-IV. Representative equilibrium-binding analyses of r-PLTP (A) and LA-PLTP (B) are presented. (■) $apoE_3$, (▲) $apoE_4$, (♦) $apoE_2$, (○) apoA-I, and (●) apoA-IV.

ApoA-IV proteoliposomes were capable of activating LA-PLTP to a similar extent as the apoE proteoliposomes (Fig. 3). Proteoliposomes containing apoA-I, however, caused no activation of LA-PLTP. Neither did apolipoprotein-free liposomes, used as a negative control, have any effect (data not shown).

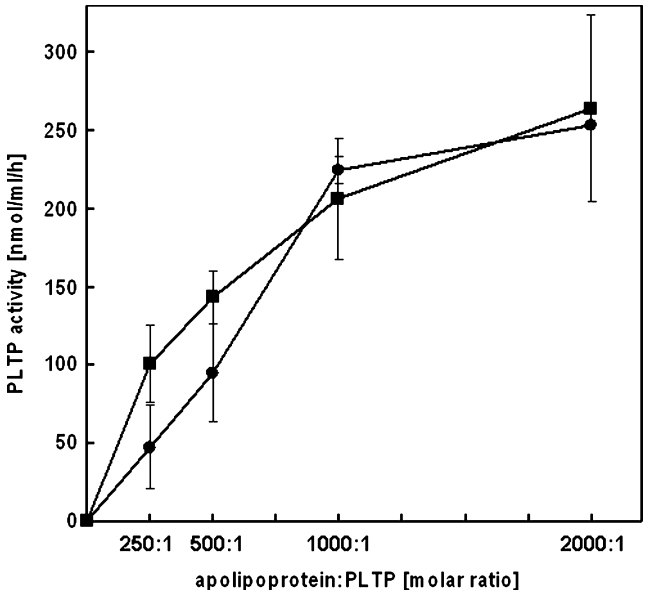

Fig. 3. Activation of LA-PLTP by proteoliposomes containing apoE and apoA-IV. The symbols represent means ± SEM for four (apoE) or three (apoA-IV) determinations. The data for apoE represent two experiments with $apoE_3$, one with $apoE_2$ and one with $apoE_4$ proteoliposomes. (■) apoE, (●) apoA-IV.

Discussion

The HDL in human plasma consists of several subpopulations of particles with distinct structure and function. This heterogeneity, which is the result of continuous remodeling of HDL by plasma factors, has important implications in terms of the cardioprotective functions of HDL. Apolipoproteins, essential in the assembly of soluble HDLs, are capable of exchanging between various classes of lipoproteins and altering their

Table 2
Binding of r-PLTP and LA-PLTP to apoE, apoA-I, and apoA-IV

Analyte	Ligand	k_a (1/Ms)	k_d (1/s)	K_D (nM)	χ^2
r-PLTP	$apoE_2$[a]	1.85 ± 1.37E+04	4.57 ± 0.56E-04	37.7 ± 25.8	3.72 ± 2.10
	$apoE_3$[a]	7.09 ± 7.77E+05	8.01 ± 1.05E-04	2.9 ± 2.7	4.31 ± 3.29
	$apoE_4$[a]	7.85 ± 8.25E+05	1.28 ± 0.69E-03	3.2 ± 2.3	2.04 ± 1.43
	apoA-I[c]	3.84 ± 3.40E+05	6.99 ± 1.27E-03	28.8 ± 15.6	2.15 ± 1.55
	apoA-IV[c]	1.51 ± 0.51E+05	2.11 ± 1.35E-02	161.0 ± 128.0	1.05 ± 0.46
LA-PLTP	$apoE_2$[b]	1.73 ± 1.19E+04	5.00 ± 2.71E-04	62.5 ± 72.4	0.13 ± 0.07
	$apoE_3$[a]	3.25 ± 3.81E+05	1.07 ± 0.08E-03	10.6 ± 10.8	0.32 ± 0.15
	$apoE_4$[a]	2.81 ± 2.73E+05	9.74 ± 2.50E-04	6.8 ± 5.0	0.40 ± 0.21
	apoA-I[c]	3.43 ± 4.29E+06	1.66 ± 0.99E-02	84.7 ± 141.1	1.23 ± 0.81
	apoA-IV[c]	1.46 ± 1.62E+06	5.28 ± 4.85E-04	1.4 ± 2.4	0.43 ± 0.48

Values are presented as mean ± SD.
The kinetic parameters were derived from measurements carried out at [a] +37 °C, [b] +25 °C or [c] +15 °C.

conformation [37]. This property is also important for their ability to interact with cellular receptors such as SR-BI and ABCA1. In addition, the interactions of apolipoproteins with enzymes such as LCAT [38] and lipoprotein lipase (LPL) [39,40], as well as the lipid transfer proteins, CETP [41] and PLTP [12,42,43], essentially contribute to the activity levels of these HDL remodeling proteins. Using surface plasmon resonance analysis we now demonstrate an interaction of PLTP with apoE, apoA-IV, and apoA-I, and show that apoE and apoA-IV, but not apoA-I, are capable of converting the inactive form of plasma PLTP into an active form.

Two forms of PLTP exist in the circulation, one active (HA-PLTP) and the other inactive (LA-PLTP). Each form is associated with different apolipoprotein–lipid complexes [14]. The physiological implications of the distribution of PLTP between HA- and LA-PLTP forms, and the mechanisms of PLTP inactivation/activation are poorly understood. Also, the exact mechanism by which PLTP mediates PL transfer is still unsolved and may possibly involve an interaction with apolipoproteins. At present, the hypothesis that PLTP functions by forming a ternary complex between donor and acceptor lipoprotein particles, thus facilitating PL transfer, is the favored one [44–46].

Studying the distribution of PLTP mass and activity in subjects with hypoalphalipoproteinemia, Oka et al. [47] demonstrated that the low plasma PLTP concentration was due to differences in the amount of inactive PLTP, while the active form was comparable to that observed in controls. In another study, infusion of apoA-I/PL discs into human subjects, despite causing a slight decrease in total PLTP mass, resulted in an increase of plasma PLTP activity [48]. These findings support the idea that, in the circulation, apolipoprotein–lipid complexes regulate the abundance of HA- and LA-PLTP, and thereby the activity of plasma PLTP.

We have previously demonstrated that PLTP binds to the N-terminal portion of apoA-I [42]. We now demonstrate using surface plasmon resonance analysis that, in addition to apoA-I, PLTP also binds to apoE and apoA-IV. An interaction of both r-PLTP and LA-PLTP with all three apolipoproteins occurred within a nanomolar K_D range, and the R_{max} values were low, indicating fast reactions of variable stability (as illustrated by the large standard deviations in Table 2). Fitting the data to different kinetic models suggests that the observed interactions do not obey a pure one site saturation model, but instead involve more complex binding dynamics possibly influenced by phospholipids associating with the PLTP proteins used. Several models, including a two-state reaction, bivalent analyte, and parallel reactions, supported the K_D values obtained using Langmuir predictions.

Although the exact PLTP binding site on apoE is currently unknown, an interesting observation is that the genetically determined isoforms, $apoE_2$, $apoE_3$, and $apoE_4$, have different affinities and binding stabilities for PLTP. Despite this, they are all capable of activating LA-PLTP. This is in accordance with our previous finding that in a Finnish population sample no differences in PLTP activity with the apoE genotypes could be demonstrated [49]. Therefore, the physiological importance of the different affinities of PLTP for the apoE isoforms remains an interesting topic for future investigation.

To determine whether the apolipoproteins that interact with PLTP are capable of converting plasma LA-PLTP to an active form, we incubated LA-PLTP in the presence of proteoliposomes containing apoE, apoA-IV, or apoA-I. To simulate the physiologic state of the apolipoproteins, they were combined with lipids for the activation experiments. The main observation from this study was that, upon incubation of the low-active form of PLTP with proteoliposomes containing apoE, an activation of LA-PLTP was evident. The ability to activate LA-PLTP is not restricted to apoE alone, as apoA-IV-proteoliposomes also induced the activation process, while proteoliposomes containing apoA-I did not. These results suggest that apoE and apoA-IV, and possibly some other apolipoproteins, play a role in maintaining plasma PLTP activity. The observation that apoA-I did not activate LA-PLTP is in line with our previous finding that apoA-I is a major component of the plasma LA-PLTP complex [14]. The present data demonstrate that plasma LA-PLTP is not purely a catabolic intermediate but instead can be converted to the active form, and suggest that the distribution of HA- and LA-PLTP is subjected to a dynamic regulation by apolipoproteins.

References

[1] W.P. Castelli, R.J. Garrison, P.W. Wilson, R.D. Abbott, S. Kalousdian, W.B. Kannel, Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham Study, J. Am. Med. Aossc. 256 (1986) 2835–2838.
[2] W.B. Kannel, High-density lipoproteins: epidemiologic profile and risks of coronary artery disease, Am. J. Cardiol. 52 (1983) 9B–12B.
[3] C.J. Fielding, P.E. Fielding, Molecular physiology of reverse cholesterol transport, J. Lipid Res. 36 (1995) 211–228.
[4] K.A. Rye, M.A. Clay, P.J. Barter, Remodelling of high density lipoproteins by plasma factors, Atherosclerosis 145 (1999) 227–238.
[5] A.R. Tall, S. Krumholz, T. Olivecrona, R.J. Deckelbaum, Plasma phospholipid transfer protein enhances transfer and exchange of phospholipids between very low density lipoproteins and high density lipoproteins during lipolysis, J. Lipid Res. 26 (1985) 842–851.
[6] J. Huuskonen, C. Ehnholm, Phospholipid transfer protein in lipid metabolism, Curr. Opin. Lipidol. 11 (2000) 285–289.
[7] X.C. Jiang, C. Bruce, J. Mar, M. Lin, Y. Ji, O.L. Francone, A.R. Tall, Targeted mutation of plasma phospholipid transfer protein

gene markedly reduces high-density lipoprotein levels, J. Clin. Invest. 103 (1999) 907–914.
[8] M. Jauhiainen, J. Metso, R. Pahlman, S. Blomqvist, A. van Tol, C. Ehnholm, Human plasma phospholipid transfer protein causes high density lipoprotein conversion, J. Biol. Chem. 268 (1993) 4032–4036.
[9] A.Y. Tu, H.I. Nishida, T. Nishida, High density lipoprotein conversion mediated by human plasma phospholipid transfer protein, J. Biol. Chem. 268 (1993) 23098–23105.
[10] C.M. Desrumaux, P.A. Mak, W.A. Boisvert, D. Masson, D. Stupack, M. Jauhiainen, C. Ehnholm, L.K. Curtiss, Phospholipid transfer protein is present in human atherosclerotic lesions and is expressed by macrophages and foam cells, J. Lipid Res. 44 (2003) 1453–1461.
[11] B.A. Laffitte, S.B. Joseph, M. Chen, A. Castrillo, J. Repa, D. Wilpitz, D. Mangelsdorf, P. Tontonoz, The phospholipid transfer protein gene is a liver X receptor target expressed by macrophages in atherosclerotic lesions, Mol. Cell. Biol. 23 (2003) 2182–2191.
[12] J.F. Oram, G. Wolfbauer, A.M. Vaughan, C. Tang, J.J. Albers, Phospholipid transfer protein interacts with and stabilizes ATP-binding cassette transporter A1 and enhances cholesterol efflux from cells, J. Biol. Chem. 278 (2003) 52379–52385.
[13] T. Oka, T. Kujiraoka, M. Ito, T. Egashira, S. Takahashi, M.N. Nanjee, N.E. Miller, J. Metso, V.M. Olkkonen, C. Ehnholm, M. Jauhiainen, H. Hattori, Distribution of phospholipid transfer protein in human plasma: presence of two forms of phospholipid transfer protein, one catalytically active and the other inactive, J. Lipid Res. 41 (2000) 1651–1657.
[14] M. Kärkkäinen, T. Oka, V.M. Olkkonen, J. Metso, H. Hattori, M. Jauhiainen, C. Ehnholm, Isolation and partial characterization of the inactive and active forms of human plasma phospholipid transfer protein (PLTP), J. Biol. Chem. 277 (2002) 15413–15418.
[15] S. Siggins, M. Jauhiainen, V.M. Olkkonen, J. Tenhunen, C. Ehnholm, PLTP secreted by HepG2 cells resembles the high-activity PLTP form in human plasma, J. Lipid Res. 44 (2003) 1698–1704.
[16] A.K. Soutar, C.W. Garner, H.N. Baker, J.T. Sparrow, R.L. Jackson, A.M. Gotto, L.C. Smith, Effect of the human plasma apolipoproteins and phosphatidylcholine acyl donor on the activity of lecithin:cholesterol acyltransferase, Biochemistry 14 (1975) 3057–3064.
[17] N. Zorich, A. Jonas, H.J. Pownall, Activation of lecithin cholesterol acyltransferase by human apolipoprotein E in discoidal complexes with lipids, J. Biol. Chem. 260 (1985) 8831–8837.
[18] T. Ohnishi, S. Yokoyama, Activation of human plasma lipid transfer protein by apolipoproteins, Biochemistry 32 (1993) 5029–5035.
[19] L.A. Main, T. Ohnishi, S. Yokoyama, Activation of human plasma cholesteryl ester transfer protein by human apolipoprotein A-IV, Biochim. Biophys. Acta 1300 (1996) 17–24.
[20] J. Huuskonen, M. Jauhiainen, C. Ehnholm, V.M. Olkkonen, Biosynthesis and secretion of human plasma phospholipid transfer protein, J. Lipid Res. 39 (1998) 2021–2030.
[21] J. Huuskonen, V.M. Olkkonen, C. Ehnholm, J. Metso, I. Julkunen, M. Jauhiainen, Phospholipid transfer is a prerequisite for PLTP-mediated HDL conversion, Biochemistry 39 (2000) 16092–16098.
[22] S. Siggins, M. Kärkkäinen, J. Tenhunen, J. Metso, E. Tahvanainen, V.M. Olkkonen, M. Jauhiainen, C. Ehnholm, Quantitation of the active and low-active forms of human plasma phospholipid transfer protein by ELISA, J. Lipid Res. 45 (2004) 387–395.
[23] J. Damen, J. Regts, G. Scherphof, Transfer of [14C]phosphatidylcholine between liposomes and human plasma high density lipoprotein. Partial purification of a transfer-stimulating plasma factor using a rapid transfer assay, Biochim. Biophys. Acta 712 (1982) 444–452.
[24] J.A. Morrow, K.S. Arnold, K.H. Weisgraber, Functional characterization of apolipoprotein E isoforms overexpressed in *Escherichia coli*, Protein Expr. Purif. 16 (1999) 224–230.
[25] S.C. Rall, K.H. Weisgraber, R.W. Mahley, Isolation and characterization of apolipoprotein E, in: J.P. Segrest, J.J. Albers (Eds.), Methods in Enzymology, Academic Press, Orlando, 1986, pp. 273–279.
[26] P.G. Lerch, J.-J. Morgenthaler, M.C. Peitsch, H. Friedli, G. Hodler, J.E. Doran, H. Isliker, H.J. Heiniger, Isolation and properties of apolipoprotein A for therapeutic use, Prot. Biol. Fluid. (1989) 409–419.
[27] L.G. Fagerstam, A. Frostell-Karlsson, R. Karlsson, B. Persson, I. Ronnberg, Biospecific interaction analysis using surface plasmon resonance detection applied to kinetic, binding site and concentration analysis, J. Chromatogr. 597 (1992) 397–410.
[28] M. Malmqvist, Surface plasmon resonance for detection and measurement of antibody–antigen affinity and kinetics, Curr. Opin. Immunol. 5 (1993) 282–286.
[29] C.E. Matz, A. Jonas, Micellar complexes of human apolipoprotein A-I with phosphatidylcholines and cholesterol prepared from cholate–lipid dispersions, J. Biol. Chem. 257 (1982) 4535–4540.
[30] C.H. Chen, J.J. Albers, Characterization of proteoliposomes containing apoprotein A-I: a new substrate for the measurement of lecithin: cholesterol acyltransferase activity, J. Lipid Res. 23 (1982) 680–691.
[31] P.J. Blanche, E.L. Gong, T.M. Forte, A.V. Nichols, Characterization of human high-density lipoproteins by gradient gel electrophoresis, Biochim. Biophys. Acta 665 (1981) 408–419.
[32] K.A. Rye, K.H. Garrety, P.J. Barter, Preparation and characterization of spheroidal, reconstituted high-density lipoproteins with apolipoprotein A-I only or with apolipoprotein A-I and A-II, Biochim. Biophys. Acta 1167 (1993) 316–325.
[33] R.J. Havel, H.A. Eder, J.H. Bragdon, The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum, J. Clin. Invest. 34 (1955) 1345–1353.
[34] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.
[35] U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature 227 (1970) 680–685.
[36] H. Towbin, T. Staehelin, J. Gordon, Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications, Proc. Natl. Acad. Sci. USA 76 (1979) 4350–4354.
[37] J. Xicohtencatl-Cortes, R. Castillo, J. Mas-Oliva, In search of new structural states of exchangeable apolipoproteins, Biochem. Biophys. Res. Commun. 324 (2004) 467–470.
[38] M.G. Sorci-Thomas, L. Curtiss, J.S. Parks, M.J. Thomas, M.W. Kearns, M. Landrum, The hydrophobic face orientation of apolipoprotein A-I amphipathic helix domain 143–164 regulates lecithin:cholesterol acyltransferase activation, J. Biol. Chem. 273 (1998) 11776–11782.
[39] R.J. Havel, V.G. Shore, B. Shore, D.M. Bier, Role of specific glycopeptides of human serum lipoproteins in the activation of lipoprotein lipase, Circ. Res. 27 (1970) 595–600.
[40] J.C. LaRosa, R.I. Levy, P. Herbert, S.E. Lux, D.S. Fredrickson, A specific apoprotein activator for lipoprotein lipase, Biochem. Biophys. Res. Commun. 41 (1970) 57–62.
[41] B.F. Asztalos, K.V. Horvath, K. Kajinami, C. Nartsupha, C.E. Cox, M. Batista, E.J. Schaefer, A. Inazu, H. Mabuchi, Apolipoprotein composition of HDL in cholesteryl ester transfer protein deficiency, J. Lipid Res. 45 (2004) 448–455.
[42] P.J. Pussinen, M. Jauhiainen, J. Metso, L.E. Pyle, Y.L. Marcel, N.H. Fidge, C. Ehnholm, Binding of phospholipid transfer protein (PLTP) to apolipoproteins A-I and A-II: location of a

PLTP binding domain in the amino terminal region of apoA-I, J. Lipid Res. 39 (1998) 152–161.
[43] P.J. Barter, O.V. Rajaram, L.B. Chang, K.A. Rye, P. Gambert, L. Lagrost, C. Ehnholm, N.H. Fidge, Isolation of a high-density-lipoprotein conversion factor from human plasma. A possible role of apolipoprotein A-IV as its activator, Biochem. J. 254 (1988) 179–184.
[44] J. Huuskonen, V.M. Olkkonen, M. Jauhiainen, J. Metso, P. Somerharju, C. Ehnholm, Acyl chain and headgroup specificity of human plasma phospholipid transfer protein, Biochim. Biophys. Acta 1303 (1996) 207–214.
[45] A. Korhonen, M. Jauhiainen, C. Ehnholm, P.T. Kovanen, M. Ala-Korpela, Remodeling of HDL by phospholipid transfer protein: demonstration of particle fusion by 1H NMR spectroscopy, Biochem. Biophys. Res. Commun. 249 (1998) 910–916.
[46] N. Settasatian, M. Duong, L.K. Curtiss, C. Ehnholm, M. Jauhiainen, J. Huuskonen, K.A. Rye, The mechanism of the remodeling of high density lipoproteins by phospholipid transfer protein, J. Biol. Chem. 276 (2001) 26898–26905.
[47] T. Oka, S. Yamashita, T. Kujiraoka, M. Ito, M. Nagano, Y. Sagehashi, T. Egashira, M.N. Nanjee, K. Hirano, N.E. Miller, Y. Matsuzawa, H. Hattori, Distribution of human plasma PLTP mass and activity in hypo- and hyperalphalipoproteinemia, J. Lipid Res. 43 (2002) 1236–1243.
[48] M.N. Nanjee, C.J. Cooke, R. Garvin, F. Semeria, G. Lewis, W.L. Olszewski, N.E. Miller, Intravenous apoA-I/lecithin discs increase pre-beta-HDL concentration in tissue fluid and stimulate reverse cholesterol transport in humans, J. Lipid Res. 42 (2001) 1586–1593.
[49] M.T. Jänis, S. Siggins, E. Tahvanainen, R. Vikstedt, K. Silander, J. Metso, A. Aromaa, M.R. Taskinen, V.M. Olkkonen, M. Jauhiainen, C. Ehnholm, Active and low-active forms of serum phospholipid transfer protein in a normal Finnish population sample, J. Lipid Res. 45 (2004) 2303–2309.