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Different Phospholipid Transfer Protein Complexes Contribute to the Variation in Plasma PLTP Specific Activity

Marian C. Cheung, Gertrud Wolfbauer, and John J. Albers

Division of Metabolism, Endocrinology and Nutrition, Northwest Lipid Metabolism and Diabetes Research Laboratories, Department of Medicine, University of Washington, Seattle, Washington, USA

Abstract

Phospholipid transfer protein (PLTP) facilitates the transfer of phospholipids among lipoproteins. Over half of the PLTP in human plasma has been found to have little phospholipid transfer activity (inactive PLTP). We recently observed that plasma PLTP specific activity is inversely correlated with high-density lipoprotein (HDL) level and particle size in healthy adults. The purpose of this study was to evaluate the factors that contribute to the variation in plasma PLTP specific activity. Analysis of the specific activity of PLTP complexes in nine plasma samples from healthy adults revealed two clusters of inactive PLTP complexes with mean molecular weights (MW) of 342kDa and 146kDa. The large and small inactive PLTP complexes represented $52 \pm 8\%$ (range 39–63%) and $8 \pm 8\%$ (range 1–28%) of the plasma PLTP, respectively. Active PLTP complexes had a mean MW of 207kDa and constituted $40 \pm 6\%$ (range 33–50%) of the plasma PLTP. The specific activity of active PLTP varied from 16 to 32 $\mu\text{mol}/\mu\text{g}/\text{h}$. These data demonstrate for the first time the existence of small inactive plasma PLTP complexes. Variation in the amount of the two clusters of inactive PLTP complexes and the specific activity of the active PLTP contribute to the variation in plasma PLTP specific activity.

Keywords

High Density Lipoproteins; Phospholipid Transfer Protein; PLTP Specific Activity; Active PLTP; Inactive PLTP

1. INTRODUCTION

Plasma phospholipid transfer protein (PLTP) is a member of the lipid transfer/lipopolysaccharide binding protein family, which also includes cholesteryl ester transfer protein, bactericidal permeability-increasing protein, and lipopolysaccharide-binding protein [1]. PLTP is a glycoprotein of diverse biological functions [2]. It can bind and transport phospholipids, free cholesterol, and α -tocopherol among lipoproteins and between lipoproteins and cells [3–5], and it can transfer lipopolysaccharide from gram-negative bacteria to lipoproteins [6]. In vitro, PLTP has been shown to induce size changes in high-density lipoproteins (HDL) [7–9], promote the efflux of cholesterol from cells [10], and

Corresponding Author: John J Albers, Ph.D, Northwest Lipid Metabolism and Diabetes Research Laboratories, 401 Queen Anne Avenue North, Seattle, WA 98109-4517, USA, Tel: 206-685-3330, Fax: 206-685-3279, jja@u.washington.edu.

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modify the anti-oxidative potentials of lipoproteins and tissues [5]. Studies using genetically engineered mice have confirmed its importance in modulating the level and composition of circulating lipoproteins and the distribution of α -tocopherol in tissues in vivo [11,12]. In PLTP-deficient mice with significantly altered vitamin E distribution in blood, phosphatidylserine (PS) externalization and PS-dependent procoagulant properties of the erythrocytes were modified [13]. There is also data on PLTP association with the prothrombinase complex assembly [14]. In human plasma, PLTP forms protein complexes with apolipoprotein (apo) A-I, apoJ, and proteins linked to immunity, inflammation, and the complement and coagulation cascades [15,16].

Two forms of functionally defined PLTP complexes have been found in human plasma. The “active” form transfers phosphatidylcholine from phospholipid vesicles to HDL, and the “inactive” form lacks this ability [17–19]. Between 55% and 90% of PLTP in plasma has been reported to be in the inactive form [18,20]. Differences in the proportion of inactive PLTP in plasma may contribute to the variation in plasma PLTP specific activity (SA). In an effort to better understand the factors that modulate plasma PLTP SA, we recently studied the plasma PLTP activity, mass, and SA of 39 healthy adults and found significant associations of these PLTP parameters with select lipoprotein classes. Importantly, PLTP SA was inversely correlated with HDL cholesterol and HDL particle size, and HDL size explained 45% of the variation of plasma PLTP SA in linear models, suggesting that HDL particle size plays an important role in modulating the relative amounts of PLTP of low and high SA in healthy men and women [21]. In this study, we sought to explore in detail the variation in the proportion of plasma PLTP that is inactive, the size distribution of the inactive and active PLTP complexes and the activity and SA of active PLTP complexes. While inactive PLTP have been reported to be in large PLTP complexes [17–19], we provide evidence for the presence of small inactive PLTP complexes and calculate the proportion of plasma PLTP in active PLTP complexes and in the large and small inactive complexes.

2. METHODS

2.1. Blood samples

To study the variation in the ability of plasma PLTP complexes to transfer phospholipids, nine subjects (4 men and 5 women) selected to represent a spectrum of HDL cholesterol levels, were recruited from the registry of the Northwest Lipid and Diabetes Research Laboratories with approval by the Human Subjects Review Board at the University of Washington. Venous blood was drawn after a 12–14 hour overnight fast. Plasma was promptly separated by low-speed centrifugation at 4°C and immediately separated on size-exclusion chromatography columns.

2.2. Size-exclusion chromatography

Plasma samples (1mL) were subjected to fast-protein liquid chromatography (FPLC) on a HiLoad™ 16/60 Superdex 200 column (GE Healthcare) at 4°C and were eluted at one mL/min with 0.01M Tris buffer containing 0.15M sodium chloride, 1mM EDTA and 0.01% sodium azide. This chromatography medium which has resolution for biomolecules in the molecular weight (MW) range of 10–600kDa was chosen to allow optimal separation of active and inactive PLTP complexes with reported mean MW of 160 and 520kDa respectively [17–19]. One ml fractions were collected and assayed for PLTP mass and activity and for cholesterol. Thyroglobulin, ferritin, catalase, aldolase, human serum albumin, ovalbumin, and cytochrome C were used as calibration proteins.

2.3. PLTP mass, activity, and specific activity

Plasma PLTP mass was measured using a double antibody sandwich enzyme-linked immunosorbent assay (ELISA) as described [21]. The assay used affinity-isolated goat-anti-PLTP as the capture antibodies, affinity-isolated chicken-anti-PLTP labeled with horseradish peroxidase as the detection antibodies, and o-phenylenediamine dihydrochloride as the substrate. Plasma phospholipid transfer activity mediated by PLTP was determined by measuring the transfer of ^{14}C -phosphatidylcholine from phospholipid liposomes to HDL using an established radioassay [22]. Plasma PLTP specific activity (SA) was calculated from the measured mass and activity data.

2.4. Other analytical procedures

Plasma cholesterol, triglyceride, direct LDL, and direct HDL cholesterol of the plasma samples fractionated on FPLC column were analyzed by enzymatic methods on a Hitachi Modular P Analytic System (Roche). ApoA-I and apoB were measured with a nephelometer (Behring Diagnostics). These analyses were performed at the Northwest Lipid Metabolism and Diabetes Research Laboratories.

3. RESULTS

3.1. Plasma contains large and small PLTP complexes with low phospholipid transfer activity

To understand the variation in the SA of plasma PLTP complexes, we analyzed the PLTP activity, mass, and SA of the PLTP complexes as a function of particle size after separation of plasma samples comprising a wide range of HDL levels (42–86 mg/dL) with size-exclusion chromatography. The recovery of PLTP mass and phospholipid transfer activity from the columns was $78 \pm 15\%$ and $95 \pm 17\%$ (mean $\pm$ S.D.), respectively. The plasma lipid profiles and PLTP mass and activity levels of the nine plasma samples analyzed are shown in Table 1, and distributions of PLTP activity, mass, and SA of their PLTP complexes as a function of size is shown in Figure 1. In all samples studied, PLTP mass was detected several fractions ahead of PLTP activity (fraction 47 or 48 vs. fraction 51–53), and all PLTP mass profiles were considerably broader than the activity profiles. This indicates that all samples contained significant amounts of large PLTP complexes that either lacked or had very low phospholipid transfer activity. In one subject, substantial PLTP mass with little phospholipid transfer activity was also detected in complexes of small size (Figure 1, last panel). In all nine samples analyzed, less than 2% of PLTP mass and activity were detected in fractions 71–80, corresponding to $\text{MW} \leq 100\text{kDa}$.

3.2. Resolution of plasma active and inactive PLTP complexes

In general, PLTP SA increased progressively from around fraction 52 (estimated $\text{MW}=410\text{kDa}$) to fraction 63 (estimated $\text{MW}=184\text{kDa}$) which likely reflects the decreased presence of large PLTP complexes containing inactive PLTP as the molecular size decreased. Note that all samples had significant PLTP mass and no PLTP activity in the very large size fractions (fractions 47 to 51). After fraction 63, PLTP SA remained relatively constant in some samples (Figure 1A, C, F) and noticeably decreased in other (Figure 1B, D, I). Plasma samples in which PLTP SA remained nearly constant after fraction 63 indicates only a minimal presence of small inactive PLTP complexes while those plasma samples in which PLTP SA decreased after fraction 63 indicate the presence of small inactive PLTP complexes accounting for the decrease in PLTP SA.

To resolve the distribution of active and inactive PLTP in the plasma, the column fraction with the highest PLTP SA in each sample analyzed was considered to contain only active PLTP, which was certainly the case in those samples with nearly constant PLTP SA in the

smaller size fractions. Fractions with PLTP SA less than peak SA level were considered to contain a mixture of active and inactive PLTP complexes. The PLTP SA of a fraction relative to peak PLTP SA thus reflects the relative amounts of active and inactive PLTP in a fraction, allowing the calculation of the mass of active and inactive PLTP in each fraction. The distribution of plasma active and inactive PLTP generated using this approach for all nine samples studied are shown in Figure 2. All plasma samples contained large inactive PLTP complexes, and small PLTP complexes with little activity were also clearly evident in some samples (Figure 2B, D, I). The mean estimated MW of the peak of the active PLTP complexes was 207 ± 7 kDa while the peak of large inactive PLTP complexes had an estimated MW of 342 ± 11 kDa and the small inactive PLTP complexes had a MW of 146 ± 12 kDa (Table 2). However, the characteristics of the mass profiles indicate that each of these three types of complexes had considerable size heterogeneity ranging from 264 to >600 kDa for the large inactive complexes, and from 119 to 170 kDa for the small inactive PLTP complexes. Large inactive PLTP complexes represented a mean of 52% (range: 39–63%) and the small inactive PLTP complexes represented a mean of 8% (range: 1–28%) of plasma PLTP in these samples, with four of the nine samples having $<5\%$ of plasma PLTP in the small inactive PLTP complexes (Table 2). Active PLTP complexes were detected within the MW range of 128–354 kDa. The SA of active PLTP complexes in the nine plasma samples varied from 16 to $32 \mu\text{mol}/\mu\text{g}/\text{h}$.

4. DISCUSSION

We have previously observed that plasma PLTP activity and mass are associated with distinct lipoprotein particles, and that HDL particle size explained 45% of the variability of plasma PLTP SA [21]. To understand the mechanisms behind the metabolic relationship between PLTP specific activity and HDL particles of large size, we sought to first understand the basis of the variation in the activity and SA of plasma PLTP. Specifically, we wanted to know if active and inactive PLTP are distinct metabolic entities that represent opposite ends of a continuous spectrum of PLTP SA, and/or if PLTP SA reflects the relative proportion of active and inactive PLTP in the plasma. We observed a prominent presence of large inactive PLTP complexes in all samples examined. After PLTP SA progressively increased as the MW of the PLTP complexes decreased (from about 401 kDa to 184 kDa), it remained relatively constant in several plasma samples (Fig 1). This strongly supports the following hypotheses: Active and inactive PLTP are distinct metabolic entities, active PLTP complexes have fairly uniform SA in a given plasma, and the variation in SA in plasma fractions as a function of size reflects the proportion of active and inactive PLTP complexes. Furthermore, it was clear that the decrease in SA in the small size fractions as they became smaller could readily be explained by the presence of small inactive PLTP complexes in these samples. Consequently, we conclude that differences in plasma PLTP SA reflect, in part, differences in the relative amount of plasma active and inactive PLTP complexes. We calculated that inactive PLTP represent $60 \pm 6\%$ of plasma PLTP in the nine samples studied. This amount agrees well with the 54% reported for a normal Finnish population [20]. Our data also confirm that inactive PLTP resides in protein complexes the size of large HDL (MW: 342 kDa) to much bigger ones (MW ≥ 600 kDa), while active PLTP resides in protein complexes the size of bulk HDL (mean MW: 207 kDa). In addition, we provide the first evidence for the presence of small inactive PLTP complexes (mean MW of 146 kDa) which constituted 1–28% of plasma PLTP.

Although we analyzed the activity, mass and SA of PLTP complexes as a function of particle size in only nine plasma samples, it is likely that our conclusions are, in general, valid for a large population, because the nine individuals studied were selected to have a broad range of HDL levels. Furthermore, these individuals also have a wide range of plasma cholesterol, triglyceride and LDL cholesterol levels representative of a larger population of

healthy adults. However, it is possible that the observations obtained with these preselected healthy adults may not be representative of individuals with gross dyslipidemia, obesity, diabetes, inflammatory conditions and other pathophysiological states. All individuals had fasted for 12–14 hours prior to blood draw. Thus, our observations do not necessarily apply to individuals in a postprandial state, nor do they provide information on the variability over time of the distribution of active and inactive PLTP complexes within an individual.

The SA of the active PLTP in the nine samples analyzed varied from 16 to 32 $\mu\text{mole}/\mu\text{g}/\text{h}$. This indicates that factors other than the amount of inactive PLTP present can influence plasma PLTP SA. Such factors could include the size and composition of the active PLTP complexes as plasma PLTP is associated with more than 20 different proteins, some of which have been shown to stabilize, enhance, or inhibit PLTP activity [16,23]. Based on the molar ratio of PLTP with other major proteins found in the PLTP complexes [16], and our estimates of the molecular size of these complexes, it is likely that there is only one molecule of PLTP per complex although, cross-linking studies of purified PLTP complexes would be needed to confirm this. In the current work further characterization of the proteins in the large and small inactive PLTP peaks from the size exclusion chromatography cannot be performed due to the presence of plasma proteins in the column fractions. However, in unpublished work we have characterized the protein content of inactive PLTP complexes removed from plasma using a monoclonal antibody (Mab4) that recognizes inactive PLTP from whole plasma [19]. Many of the proteins associated with the inactive PLTP complexes isolated using Mab4 are found also in the active PLTP complexes [16] but the relative proportions of the proteins in the complexes differ significantly between active and inactive complexes. Whether or not compositional differences between active and inactive complexes account for the lack of phospholipid transfer activity in inactive complexes remains to be established, but this is the most plausible explanation. Furthermore, we have shown that PLTP complexes contain very little lipid [16]. Thus, the amount of lipid per PLTP particle presumably varies little and the variation in PLTP complex size is likely due primarily to the nature and number of protein molecules in the PLTP complexes rather than to differences in the lipid /protein ratio of the complexes.

HepG2 cells are known to produce only nascent HDL particles and conditioned medium from these cells appears to contain only the active form of PLTP [24]. As active PLTP is associated with relatively small apolipoprotein A-1 / PLTP complexes, and the inactive form is located predominantly on large PLTP complexes [17–19, and this study], we hypothesize that inactive PLTP is generated in the vascular compartment during the conversion of nascent apolipoprotein A-1 / PLTP containing complexes to large PLTP complexes. Inactive PLTP could be formed as proteins are transferred from apolipoprotein B-containing lipoproteins or cells to nascent PLTP complexes. The lack of correlation between PLTP activity and mass [21,25,26] suggests that plasma PLTP activity can increase or decrease without a parallel change in PLTP protein. To explain this phenomenon, we further hypothesize that inactive PLTP can regain its phospholipid transfer activity when large PLTP complexes are catabolically converted into active smaller PLTP complexes. Interestingly, the current study shows that although the majority of small PLTP complexes are active, some are clearly inactive. The proposed inter-conversion of PLTP with low and high specific activity could be a mechanism through which PLTP activity and consequently the pool size of active and inactive PLTP is regulated *in vivo*.

Some future directions of research include exploring the factors that alter the level and size distribution of active and inactive PLTP, document that the conversion of inactive to active PLTP and *vice versa* occurs and assess the factors that regulate these conversions, and further evaluate the potential physiological role of inactive PLTP complexes. Inactive PLTP complexes may act as a plasma reservoir of PLTP that can be rapidly converted to active

PLTP as needed. However, inactive PLTP could also play other important physiological roles. For example, we have previously reported that PLTP, like apoA-1, can bind the ABCA1 transporter and consequently activate Janus kinase 2 [27]. We have also shown that a mutant PLTP M159E that does not have the ability to transfer phospholipid from liposomes to HDL, still has the capacity to bind ABCA1 and activate Janus kinase 2 [28]. Thus, inactive PLTP complexes may retain their ability to activate the Janus Kinase 2/Stat 3 pathway. More studies are needed to evaluate the relative roles active and inactive PLTP play in the regulation of intracellular pathways.

In summary, this study demonstrates the presence of two clusters of inactive PLTP complexes, one large and one small. The variation in the amount of the two inactive PLTP clusters, along with variation in the specific activity and amount of active PLTP complexes, contributes to the variation in total plasma PLTP specific activity.

Acknowledgments

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Figure 1.

PLTP activity, mass, and specific activity profiles in the plasma samples of subjects 1 to 9 (panels A–I, respectively). One ml plasma sample from each subject was separated by FPLC using a HiLoad™ 16/60 Superdex 200 column. One ml column fractions were collected and assayed for PLTP activity (open circle) and mass (open triangle), and PLTP specific activity (closed square) was calculated. In panel I, the plasma cholesterol profile (x) showing the elution position of VLDL+ LDL (first peak), and HDL (second peak) was also included for reference. PLTP and cholesterol are expressed as percentage of total activity or mass in all column fractions.

Figure 2.

Distribution of active (open circle) and inactive (closed circle) PLTP complexes in the plasma samples of subjects 1 to 9 (panels A–I, respectively). PLTP SA of each column fraction as shown in Figure 1 was used to resolve the mass of active and inactive PLTP in each fraction. The column fraction(s) with the highest PLTP SA in each plasma sample was considered to contain only active PLTP.

Table 1

Lipid profiles of the subjects

Subject	Gender	CH	TG	VLDL-C	LDL-C	HDL-C	HDL ₂ -C	HDL ₃ -C	A-I	B
1	M	199	169	34	124	41	6	35	125	84
2	M	166	89	18	105	43	8	35	125	80
3	F	210	160	32	132	46	7	39	127	104
4	F	175	122	24	99	52	15	37	166	93
5	M	222	97	19	149	54	8	46	163	125
6	F	251	151	30	160	61	18	43	172	154
7	M	261	59	12	187	62	12	50	139	129
8	F	192	43	9	106	77	32	45	185	73
9	F	193	53	11	96	86	33	53	195	64

All values are expressed in mg/dL. The subjects were chosen to represent a range of plasma HDL cholesterol level.

Table 2

Level and molecular size of plasma active and inactive PLTP complexes

Subject	Total Plasma PLTP			Active PLTP			Large Inactive PLTP			Small Inactive PLTP		
	Activity μmol/ml/h	Mass μg/ml	SA μmol/μg/h	Mass (μg/ml)	Fraction (% of Total)	MW (kDa)	Mass μg/ml	Fraction (% of Total)	MW (kDa)	Mass μg/ml	Fraction (% of Total)	MW (kDa)
1	12.8	1.26	10.21	0.45	35.8	205	0.79	63.0	341	0.02	1.2	142
2	14.0	1.74	8.05	0.75	43.2	198	0.83	47.8	329	0.16	9.1	137
3	9.7	1.36	7.13	0.49	36.1	198	0.82	60.1	329	0.05	3.8	123
4	10.3	1.30	7.97	0.59	45.4	221	0.58	44.5	354	0.13	10.0	148
5	16.6	1.77	9.35	0.88	49.5	212	0.79	44.5	341	0.11	5.9	159
6	13.9	1.69	8.18	0.75	44.4	205	0.88	51.7	341	0.07	3.9	159
7	21.1	1.68	12.59	0.65	39.0	205	0.94	56.0	341	0.08	5.0	159
8	11.8	1.60	7.35	0.53	33.2	205	1.02	63.3	341	0.06	3.6	148
9	15.5	3.00	5.15	0.99	32.8	212	1.18	39.4	363	0.84	27.8	142
Mean	14.0	1.71	8.44	0.68	39.9	207	0.87	52.3	342	0.17	7.8	146
S.D.	3.5	0.52	2.10	0.18	5.9	7	0.17	8.8	11	0.25	8.0	12