Phospholipid Transfer Proteins and their Regulatory Role in Lipid Metabolism and Potential Biological Functions

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

Review Article

ABSTRACT

The phospholipid transfer protein (PLTP) is a lipid transfer glycoprotein that binds to and transfers a number of amphipathic compounds. In previous studies, the focus of the scientific community was on the specific role of PLTP in high-density lipoprotein (HDL) metabolism. Both PLTP and related cholesteryl ester transfer protein (CETP) are secreted proteins, and adipose tissue is an important contributor to the systemic pools of these two proteins. PLTP activity and its mRNA can be regulated by several factors. A diet rich in high-fat cholesterol results in a substantial increase in PLTP activity and in its mRNA levels. After a lipopolysaccharide injection, plasma PLTP activity is significantly reduced, and this is associated with a similar reduction in PLTP mRNA levels in the liver and adipose tissues. PLTP expression and activity can also be affect by glucose and insulin. This review article examines recent advances in the understanding of its potential biological roles.

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1. INTRODUCTION

Phospholipid transfer proteins (PLTP) is a member of a family of lipid transfer/ lipopolysaccharide-binding proteins, including cholesterol ester transfer protein (CETP), lipopolysaccharide-binding protein (LBP) and bactericidal/permeability increasing protein as well as phosphatidylinositol proteins (PITP) as depicted in Fig. 1. Besides the phospholipids, PLTP also efficiently transfers diacylglycerol, α-tocopherol, cerebroside, and lipopolysaccharides. Thus, the plasma PLTP is not a specific lipid transfer protein. It has also been reported that there are two forms of lipoprotein-associated PLTP proteins [1]. Molecular cloning of a cDNA from endothelial and active plasma cells has already been done [2]. The PLTP is is known to be associated with apoA-I containing lipoproteins of about 160 kDa in size and inactive one is associated with apoE-containing lipoproteins of about 520 kDa in size [3,4]. In phospholipid break down signal transduction the phosphatidyl inositol (PI) and to some extent phosphatidylcholine (PC) play an important role. The three known soluble PLTPs are PITPα, PITB β and RdgB proteins, RdgBα1 also known as PITPNM1; Nir2), RdgBα2 also known as PITPNM2; Nir3, and RdgBβ (PITPNC1), belong to a less well characterized with respect to their lipid binding properties [5]. Studies conducted on the structure of PITPα containing PI, suggest four residues (Thr-59, Lys-61, Glu-86, and Asn-90) which are important for PI binding in PITPα [6]. These residues are normally conserved in the entire PITP family, and any mutation of these residues in PITPα can lead to a loss of PI binding. Despite the availability of the structures of PITPα and of PITPβ containing PC, residues responsible for PC binding have not been easy to identify. It has recently been shown that the mutation of Cys-95 to threonine results in a loss of PC transfer in PITPβ [7]. This review encompasses the roles of PLTPs in intracellular lipid, cholesterol and phospholipid trafficking as well its exogenous biological roles.

2. PHOSPHOLIPID TRANSFER PROTEINS AND ATHEROSCLEROSIS

Recent studies suggest that PLTP α is upregulated in atherosclerosis and it is possible that suggestions of positive association between PLTPα and acute cardiovascular disease (CVD) reflect sampling of subjects already with significant atherosclerotic disease, and therefore, higher PLTPα, which may be a consequence of the underlying pathophysiological processes or a compensatory change [8]. In addition, it may be that the cardioprotective effects of PLTP α have increased importance in primary prevention i.e., before development of sizable atherosclerotic plaque due to its important role in reverse cholesterol transport. Notably, the prospective work by Robins *et al* found out that higher levels of PLTP α were associated with incident CVD; however, the measures of PLTP α were from the fluorometric - based assay and did not specifically assess the cerebrovascular disease, instead using the composite outcome CHD or hemorrhagic stroke or ischemic stroke as the incident CVD [9,10]. As a result, it is perceived that further prospective studies beginning with disease-free participants and measuring PLTPα with the radiometric-based, standardized direct transfer method are needed to firmly establish whether PLTP α is causally protective against cerebrovascular disease including coronary artery disease (CAD) in particular [11. Fig. 2 describes the immunoblotting of a prototype PLTP.

3. PLTP AND TRANSPORT OF PHOSPHOLIPIDS

Mammals are known to express two isoforms of the phosphatidylinositol (PI) transfer protein, PITPα and PITPβ [7]. The PI transfer proteins are soluble, cytosolic proteins that bind and transfer PI and PC between membranes *in vitro*. The inter-membrane transfer of PI by these proteins is about 10–20 times more faster than for the PC [12]. Interestingly, the mammalian PI transfer protein is highly homologous to the yeast Sec14 protein that is essential for budding of secretory vesicles from the Golgi because in Sec14-deficient yeast the PC content of the Golgi apparatus is increased and protein secretion is impaired [13]. The mammalian PI transfer protein is also known to associated with Golgi membranes, indicating that this protein might play a role in vesicle transport/protein secretion in mammalian cells. Additional studies report that mammalian PI transfer protein transfers newly made PI from the ER to the plasma membrane for conversion to the phosphoinositides used in signaling pathways; however, confirmation of this function of PI transfer protein is still required. Nevertheless, the mammalian PI transfer proteins do play important roles that have not yet been completely elucidated. For example, PITPα mice die shortly after birth due to severe neurodegeneration. Moreover, elimination of PITP β from mice is lethal to the embryo [14].

However, evidence that mammalian PI transfer proteins act as inter-organelle PC and/or PI transport proteins *in vivo* is still scatchy and many questions remain about their potential biological functions.

4. PLTP AND CHOLESTEROL TRANSPORT

PLTPs appear to function in the intermediary transfer of excess cellular lipids to lipoproteins through its interaction with ATP binding cassette transporter (ABCA1) [15]. It was also indicated that an amphipathic helical region of the N-terminal barrel of PLTP is imperative for ABCA1-dependent cholesterol efflux. Furthermore, Lee-Rueckert et al. [16] studied the ABCA1-dependent efflux of cholesterol from peritoneal macrophages obtained from PLTP-deficient mice and compared it with cholesterol efflux from wild-type macrophages. They found that cholesterol efflux from PLTP-deficient macrophage foam cells is defective and that the defect can be corrected by robust stimulation of the ABCA1-dependent pathway [17]. Altogether, results supported an intracellular role for endogenous macrophage PLTP in ABCA1-mediated cholesterol efflux from macrophage foam cells. As mentioned previously, PLTP is present in plasma as two forms, a highly active (HA-PLTP) and a lowly active (LA-PLTP) form. Vikstedt et al. [18] reported that incubation of HDL in the presence of HA-PLTP resulted in the formation of preβ-HDL and resulted in 42% increase in macrophage cholesterol, while LA-PLTP neither formed preβ-HDL nor increased cholesterol efflux [19]. However, neither HA- nor LA-PLTP enhanced cholesterol efflux to lipid-free apoA-I. Therefore it can stated based on the above results, that PLTP may promote macrophage cholesterol efflux [20].
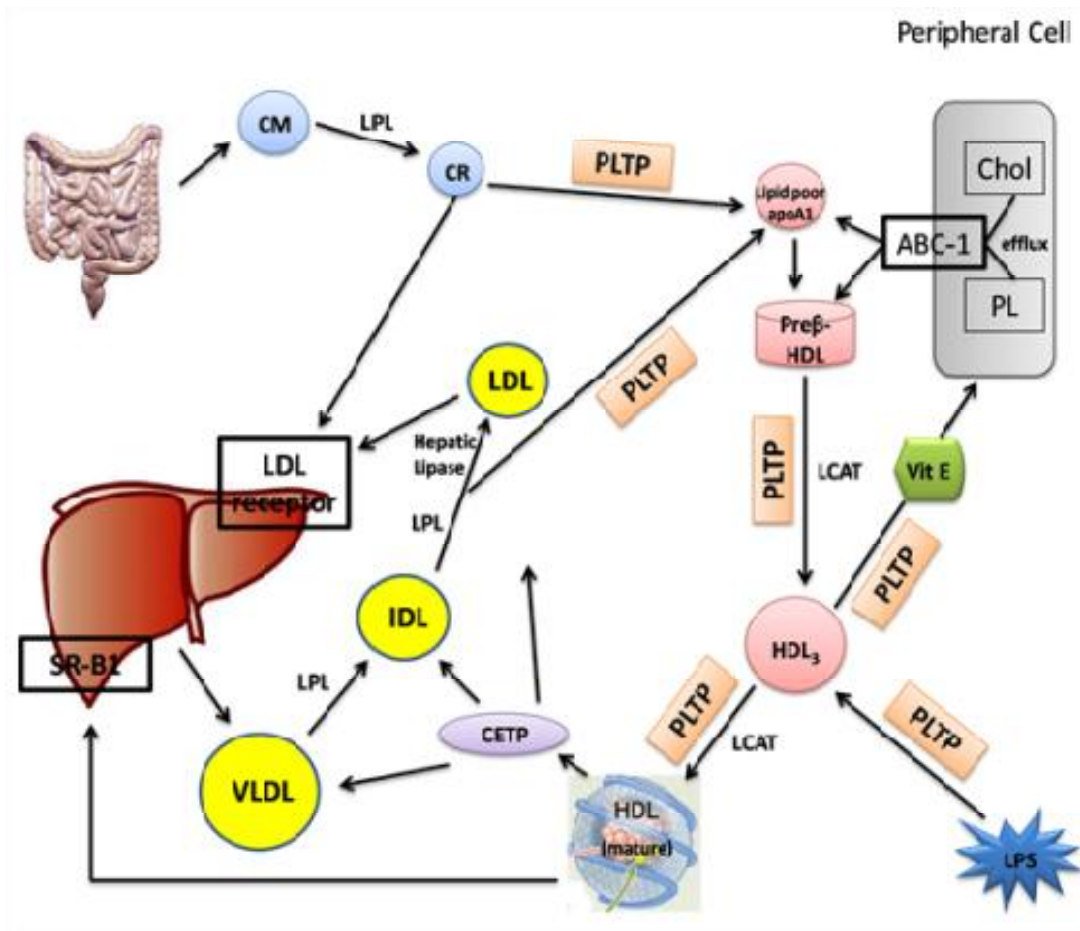

Fig. 1. Role of PLTP in lipid metabolism
Fig. 1 Courtesy: Ian James Martins and Rhona Creegan, Health, Vol. 6(12), 2014

5. PLTP BINDING TO PHOSPHATIDIC ACID AND IMPACT ON PHOSPHOLIPASE D OR C ACTIVITY

Recent studies provide enough evidence that the PITP domain of Class II PITPs can bind PA in addition to binding PI and PC. Thus, the lipid binding properties of Class II PITPs are different from those of Class I PITPs. This in itself is expected given that these PITP domains only share ~40% identity in their amino acid sequence. Residues that are essential for binding the inositol headgroup of PI are conserved in all PITPs, and therefore PI binding and transfer are shared by all PITPs. As residues important for PC binding and transfer are less obvious despite the availability of the structures of both PITPα and PITPβ loaded with PC, it has not been possible to predict whether RdgBβ will also be competent for PC binding and transfer. These studies examined the lipid binding and transfer properties of RdgBβ. Recent experimental studies show that although all members of the PITP family can bind and transfer PI the Class IIB PITP, RdgBβ, depicts a drastically reduced PC binding and transfer activity. Using HL60 cells prelabeled with [^{14}C]acetate as lipid donors, RdgBβ was found to bind mainly PI and PA [21]. In contrast, PITPα was bound to only PC and PI. PA binding by RdgBβ has been confirmed by mass spectrometry, which also depicts that among the cellular PA species RdgBβ preferably binds the C16:0/16:1 and C16:1/18:1 PA species. These PA species are typically produced from PC via the PLD pathway [22]. Using specific inhibitors for PLC or PLD enzymes it was confirmed that the PA bound by RdgBβ is mainly derived from the PLD pathway. The ability to bind PA has been shown to be conserved in all members of the Class II PITPs. However, RdgBβ was bound to the maximum level with PA.

6. RDGBβ AN IMPORTANT PLTP

Comparativey little is known about the smaller, soluble RdgBβ protein. Two variants of RdgBβ have been described according to splicing of transcripts; a long splice variant, referred to as RdgBβ (-sp1) (332 amino acids, 38 kDa) and translated from an mRNA comprised of exons 1-8 and 10 and the short splice variant, sp2 (268 amino acids, 32 kDa) translated from an mRNA comprised of exons 1-9. Expression of RdgBβ has been examined at the mRNA level and transcripts are enriched in the heart, muscle, kidney, liver, and peripheral blood leukocytes,

brain and testes [23]. However, the presence of the endogenous protein has mainly been described from high-throughput proteomic screens. Large scale phosphoproteomic studies have reported that the C-terminus of RdgBβ is phosphorylated [24,25] *in vivo* at Ser274 and Ser299. Phosphoproteomic analysis of the developing mouse brain (fore-brain and mid-brain from E16.5) and HeLa cells in G1 phase of the mitotic cycle identified Ser274 as being phosphorylated. Ser299 was found to be phosphorylated in human embryonic stem cells [26], following receptor tyrosine kinase stimulation of cancer cell lines (H1703, H3255 and MKN45 cells) by EGF, PDGF and c-Met, after stimulation of signalling by the oncogenic mutant of Flt3, as well as in Jurkat cells treated with phosphatase inhibitors calyculin and pervanadate [27-29]. RdgBα proteins also bind PA, it is possible that they are responsible for PA transfer at intermembrane contact sites. Because PA can be metabolized into DAG by phosphatidic acid phosphodyrolase enzyme, RdgBα proteins could possibly modulate the availability of DAG. In *C. elegans*, mutations in DAG kinase can rescue the phenotypes of RdgBα loss of function mutants. In Drosophila photoreceptors, the Dm-RdgBα proteins are thought to be localized at the subrhabdomeric region as is the single phospholipase D [21], whereas the hydrolysis of PI(4,5)P$_2$, resulting in the generation of DAG, occurs at the nearby but distinct microvillar plasma membrane. In this context, the movement of lipids between the microvillar plasma membrane and the subrhabdomeric region needs to be maintained so that the PI(4,5)P$_2$ cycle is functional, and the possibility that RdgBα proteins could possibly facilitate the PA removal from the rhabdomere [30].

7. TECHNIQUES EMPLOYED IN STUDIES ON PLTP

7.1 PLTP mRNA Quantification

Total RNA can be isolated from cultured cells for e.g porcine brain endothelial cells (pBCEC) or rat lung type II cells using RNeasy mini kit according to the protocol of the manufacturer, and RNA integrity is evaluated by 1% agarose gel electrophoresis. RNA concentration is determined spectrophotometrically, and total RNA (1g) is reverse-transcribed using Script cDNA synthesis kit (Bio-Rad) on a C1000 Thermal Cycler (Bio-Rad). Quantitative gene expression analysis of PLTP and reference genes HPRT1 (hypoxanthine phosphoribosyl-

transferase 1), ACTB (β-actin), GAPDH (glyceraldehyde-3-phosphate dehydrogenase), TBP (TATA box-binding protein), RPL4 (ribosomal protein L4), and HMBS (hydroxymethylbilane synthase) are performed on a CFX 96 Real Time System (Bio-Rad) using SYBR Green technology. In general, each reaction (10 µl) contained 1 iQ SYBR Green Supermix (Bio-Rad), 300nM of each primer as shown in Table 1 and 20 ng of cDNA template; PCR cycling conditions consisted of 40 cycles at 95°C for 20 s, 60°C for 40 s, and 72°C for 40 s. All reactions are run in triplicate, and melting curve analyses are routinely performed to monitor the specificity of the PCR product. The relative gene expression ratio is determined using a standard curve method.
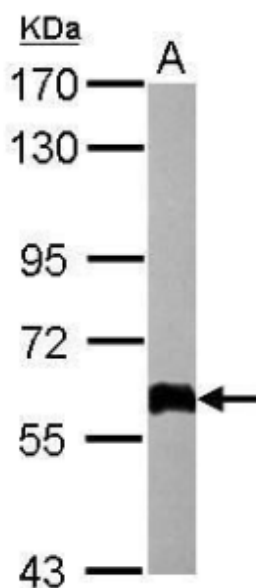

Fig. 2. Immunoblotting analysis of phospholipid transfer protein
(Picture courtesy: Antibodies online.com, USA)

7.2 Measurement of Phospholipid Transfer protein Activity

Phospholipid transfer activity of PLTP can be estimated based on the transfer of L [^{3}H] dipalmitoylphosphatidylcholine from liposomes to High density lipoprotein subfraction 3, (HDL3) using an established radiometric assay. Egg PC in concentration of 129 µmol/µl, 1 nmol/l butylated hydroxytoluene, and 1 µCi/µl L--[^{3}H]dipalmitoylphosphatidylcholine are dried under nitrogen and resuspended in 1 ml of substrate buffer (10mMTris-HCl, 150 mM NaCl,

and 1 mM EDTA, pH 7.4). To obtain clear liposomes, the above solution is sonicated and centrifuged (11,000 g, 12 min, at room temperature). Radio-labeled liposomes (150 nmol) and plasma HDL3 (200 g) are incubated with aliquots of pBCEC supernatants (300 µl) or cellular lysates (100 µl; in substrate buffer) for 1 hour at 37°C. The reaction is stopped, and liposomes are selectively precipitated by the addition of stop solution (536 mM NaCl, 363 mM MnCl$_2$, and 52 units of heparin). After incubation for 30 min on ice, the mixture was centrifuged (12,000 g for 10 min) at room temperature, and the resultant radioactivity transferred to HDL3 in the supernatant (500 µl) was determined on a Tri-Carb 2100 TR Liquid Scintillation Counter (Packard Bioscience Co.) after mixing with 5.0 ml of Ultima Gold scintillation mixture.

7.3 Role of Next Generation Proteomics

The next generation proteins can affect the function of these transfer proteins and can be of use clinically. By constraining the peptide, or for that matter the multiple peptides, by using a large protein structure i.e 'scaffold' protein can produce better affinities and specificities and starts to mimic the way that the variable region of an antibody presents peptide sequences. The development of robust molecular display technologies has resulted in controlled approaches in the development of protein scaffolds. These experiments are carried out completely *in vitro*, and they overcome the limitations of the immune system and allow for the conditions of experimentation to suit the demands of the target in the generation of specific binders. Several companies have brought different proprietary scaffold proteins to the market, almost always derived from naturally occurring small proteins or protein fragments ranging in MW from 7-19 kDa [31]. The scaffold proteins have been engineered in a way that loops or surfaces with variable peptide insert lengths to create specific binding sites with the target molecule. Most of this technology has been targeted at the development of therapeutics with many scaffold proteins now in the final phases of clinical trials or having been licensed for use in diagnosis or therapeutics. Scaffold proteins could help in modulating the actions of phospholipid transfer proteins by using protein-protein interactions, thus can have potential role in diagnostics use as biomarkers (atherosclerosis and cardiovascular diseases) and therapeutics [32].

Table 1. Various primer sequences used for real time PCR

Gene	NCBI gene ID or ref.	Primer sequence (5′–3′)	Amplicon length (bp)
1) ssPLTP	397527	F,CCCTCTTCCTAGTGCTGCTG R,CAGATCCGGAATGGTAATGG	146
2) ssHMBS	27	F, AGGATGGGCAACTCTACCTG R, GATGGTGGCCTGCATAGTCT	83
3) hsPLTP	26	F,CAGGCGCACATGCAGAGTTC R, GCAGCTCTGTGACCTTCACC	183
4) sBACE1	100511707	F, TGGACTGCCTCATGGTGTG R,GTGACCAAAGTGAACCACCG	155
5) hsHMBS	3145	F, TGCTCGCATACAGACGGAC R,GGTAAACAGGCTTTTCTCTCCAA	111

8. CONCLUSION

In the recent years considerable progress has been made as far as the structural aspects of the PLTPs are concerned. However much work still is required to elucidate the exact biological importance of these proteins although there is progress in this regard. New insights must focus on the possible interaction of the various different PLTPs with signal transducing molecules and whether they can be of use as biomarkers or targets for therapeutics. One of the important tool in elucidating these mechanism would be the use of proteomics technology.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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