The role of binding in the lipid transfer process

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Richard E. Morton

From the Department of Cardiovascular Research, Atherosclerosis Section, The Cleveland Clinic Foundation, Cleveland, Ohio 44106

Plasma-derived lipid transfer protein (LTP) facilitates the transfer of cholesteryl ester and triglyceride between all lipoproteins. Kinetic models of the transfer event have suggested that transfer is facilitated through the physical interaction (binding) of LTP with its lipoprotein substrate. Such binding has been observed previously between LTP and high density lipoprotein (HDL), but not between LTP and low (LDL) or very low (VLDL) density lipoproteins. In the present study, the interaction of LTP with plasma lipoproteins has been re-evaluated. These experiments have employed Sepharose-bound lipoproteins in order to facilitate the rapid separation of unbound and lipoprotein-associated LTP. The validity of this approach in assessing LTP-lipoprotein interactions was evidenced by the fact that free (unbound) lipoproteins could competitively inhibit or disrupt the binding of LTP to the Sepharose-bound lipoproteins. LTP was observed to bind to VLDL, LDL, and HDL. Whereas VLDL- and LDL-LTP complexes were labile and almost completely dissociated in 90 min, HDL-LTP complexes remained intact during this time. Under equilibrium conditions, LTP binding to all lipoproteins was characterized by high affinity, saturable kinetics; the apparent affinities (Kd) of VLDL, LDL, and HDL for LTP were nearly the same (25 nM). The results of two studies correlated lipid transfer activity with LTP binding to lipoproteins: 1) LTP binding and transfer activity increased in parallel as the amount of LTP in the assay was increased and 2) the inhibition of transfer activity caused by differing amounts of an inhibitor protein correlated with similar decrements in LTP binding. The latter data also suggest that the inhibitor protein suppresses lipid transfer activity by disrupting LTP-lipoprotein interactions. It is concluded that LTP avidly binds to VLDL, LDL, and HDL via a reversible, saturable mechanism and that the binding of LTP to the lipoprotein surface is an integral component of the lipid transfer reaction.

Zilversmit et al. (1) were the first to demonstrate that plasma contains a specific protein (or proteins) which facilitates the transfer/exchange of cholesteryl ester between iso-

lated lipoproteins. Subsequently, several laboratories (2-4) have purified a protein from human plasma which is responsible for the majority of core lipid transfer activity. This protein, designated LTP, is characterized as a hydrophobic glycoprotein of 58,000-63,000 molecular weight and an isoelectric point of 5.2 (2-4). Purified LTP facilitates the transfer of cholesteryl ester, triglyceride, and phosphatidylcholine (3). Cholesteryl ester and triglyceride appear to compete for transfer, with the extent of transfer for each lipid being determined by its relative concentration in the donor lipoprotein (5). Through this competition mechanism, LTP can facilitate both the homoexchange (i.e. cholesteryl ester for cholesteryl ester and triglyceride for triglyceride) and the heteroexchange (i.e. cholesteryl ester for triglyceride) of lipoprotein core lipids. The latter process facilitates the net transfer of cholesteryl ester and triglyceride between lipoproteins, which is an important step in the catabolism of very low density lipoprotein to low density lipoprotein (6).

Whereas the function of LTP is partially elucidated, little is known about the molecular mechanism by which the transfer process occurs. Two kinetic models have been proposed to describe the lipid transfer reaction. In the model described by Barter et al. (7), LTP is a carrier molecule which binds lipid and then shuttles it between lipoproteins. In contrast, the data of Ihm et al. (8) suggest that LTP mediates the formation of a ternary complex of donor lipoprotein, acceptor lipoprotein, and LTP; thus, LTP facilitates lipid transfer via this trimolecular complex. Although these models are mechanistically different, both models propose that LTP binds to the lipoprotein surface in order to facilitate lipid transfer. LTP has been shown to bind to high density lipoprotein, but no measurable interaction between LTP and low or very low density lipoproteins has been detected (9). However, since LTP readily facilitates lipid transfer between isolated very low and low density lipoproteins, it follows that LTP must also bind to these lipoproteins if either of these models is correct. In the present study, the interaction of LTP with plasma lipoproteins has been reinvestigated under conditions which are more favorable to the detection of transient LTP-lipoprotein complexes than those conditions previously employed (9). The importance of LTP-lipoprotein interactions to the lipid transfer reaction itself has also been evaluated.

Experimental Procedures

Materials—Glycerol tri[9,10-3H]oleate (470 mCi/nmol) and [4-14C]cholesterol (57.8 mCi/nmol) were purchased from Amersham. [14C]

The abbreviations used are: LTP, lipid transfer protein; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; IDL, intermediate density lipoprotein; BSA, bovine serum albumin.
Lipid Transfer Protein

Cholesterol oleate was synthesized from [4-14C]cholesterol and oleoyl chloride (Sigma) as described by Pinter et al. (10). Triglyceride and cholesterol ester with initial chemical purities of >98% were purified by thin layer chromatography on Si60 plates (E. Merck, Darmstadt, Germany) in a developing system of hexane/diethylether (80:20, v/v) that contained room temperature.

Table I

<table>
<thead>
<tr>
<th>Lipid Transfer Activity</th>
<th>Activity bound</th>
<th>Cholesterol ester transfer activity bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL-Sepharose</td>
<td>71.4</td>
<td>69.9</td>
</tr>
<tr>
<td>LDL-Sepharose</td>
<td>94.3</td>
<td>92.7</td>
</tr>
<tr>
<td>HDL-Sepharose</td>
<td>97.6</td>
<td>97.0</td>
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</table>

RESULTS

In initial studies, we confirmed the observations of Pattnaik and Zilversmit (9) which demonstrated that, as analyzed by gel filtration, LTP binds to HDL but apparently not to VLDL or LDL. Since the gel filtration approach used here and by Pattnaik and Zilversmit required 4-6 h for the separation of free and lipoprotein-associated LTP, it seemed reasonable that the binding of LTP to VLDL or LDL may have been obscured by the dissociation of these complexes during the gel filtration procedure. To facilitate more rapid separation of free and bound LTP, Sepharose-bound VLDL, LDL, and HDL were prepared; these solid phase lipoproteins were packed into columns and eluted under conditions that separated free LTP from putative LTP-lipoprotein complexes in <5 min. Using this approach, the binding of LTP to all plasma lipoproteins was observed (Table I). Columns of LDL- and HDL-Sepharose bound the applied LTP almost quantitatively...
and VLDL-Sepharose bound ≈70%. Sepharose alone accounted for <10% of the measured binding. Both triglyceride and cholesterol, ester transfer activities were bound to an equal extent, consistent with the transfer of these lipids by a common protein (3, 4); the lipid transfer activity reported in subsequent experiments refers to cholesteryl ester transfer alone. Although partially purified LTP was applied to the columns, the specificity of protein binding to the solid phase lipoproteins was evidenced by the fact that <5% of the applied protein was retained under conditions where LTP was almost quantitatively bound. This small fraction of protein binding is consistent with calculations which indicate that ≈4% of the applied protein is LTP (5).

Partially purified LTP was not unique in its ability to bind to the solid phase lipoproteins. When freshly isolated, whole human plasma was rapidly eluted through columns of LDL- or HDL-Sepharose, more than 50% of the endogenous lipid transfer activity was bound (Table II). Under these conditions, there was no significant loss of protein or cholesterol from the eluted plasma sample, showing that the binding of LTP to the lipoprotein-Sepharose columns was not a reflection of generalized protein or lipoprotein loss. Thus, LTP in whole plasma, which may be associated with other plasma proteins (26, 27), and partially purified LTP are similar in their ability to bind to lipoproteins.

The stability of LTP and lipoprotein-Sepharose complexes was investigated in order to reconcile the apparent discrepancy between the results in Tables I and II and the previously published experiments which found no apparent interaction between LTP and VLDL or LDL (i.e., the gel filtration studies). LTP was applied to columns of VLDL, LDL, or HDL-Sepharose and the column eluates were monitored continuously for dissociated lipid transfer activity. Consistent with expectations based on gel filtration data (9), there was no detectable dissociation of LTP-HDL Sepharose complexes over a 90-min period (Fig. 1). In contrast, LTP-LDL and LTP-VLDL complexes were stable for ≤15 min, then rapidly and almost completely dissociated over the test period. These results indicate that the binding of LTP to LDL and VLDL is sufficiently labile as to have been undetectable by the gel filtration technique.

The interactions between LTP and lipoprotein Sepharose were studied by measuring the ability of free (non-Sepharose bound) lipoproteins to compete for LTP binding. When lipoproteins were premixed with LTP and then applied to lipoprotein-Sepharose columns, the binding of LTP to VLDL-, LDL-, and HDL-Sepharose was progressively inhibited by increasing amounts of VLDL, LDL, or HDL, respectively (Fig. 2). Under these rapid flow conditions, an amount of free HDL equal to that present in the HDL-Sepharose column, reduced LTP binding by 70%; an equal amount of LDL inhibited LTP binding to LDL-Sepharose by 55% and an equal amount of VLDL inhibited binding to VLDL-Sepharose by 60%. At higher lipoprotein concentrations, the binding of LTP to the solid phase lipoproteins could be reduced to levels near zero. In a separate experiment (data not shown), free lipoproteins were equally or more effective in displacing LTP which had been previously bound to HDL- or LDL-Sepharose as they were in inhibiting the binding when LTP and lipoprotein were applied together. Collectively, these results show that LTP binding to Sepharose-bound lipoproteins is comparable to an interaction that also occurs between LTP and soluble lipoproteins, i.e. the binding of LTP to lipoprotein-Sepharose gels is not the consequence of the lipoprotein-Sepharose conjugation. In addition, the LTP associated with HDL-Sepharose is not bound irreversibly, as may be implied from the fact that HDL-Sepharose-LTP complexes are long lived and can be isolated (9), but rather there is very rapid equilibration of LTP between free and Sepharose-bound lipoproteins.

Whereas the above experiments employed columns of lipoprotein-Sepharose with high flow rates (i.e. short contact times between applied LTP and column matrix) to measure LTP-lipoprotein interactions, the kinetics of LTP binding to plasma lipoproteins were studied by incubating LTP with a mixture of lipoprotein-Sepharose under equilibrium conditions. Preliminary experiments indicated that binding was at equilibrium in ≈2 h at 25°C; 3 h was used as the experimental period. Based on the foregoing results, it appears that this long equilibration time reflects the slow diffusion of LTP into the gel matrix. Under these conditions, the binding of LTP to VLDL, LDL, and HDL demonstrated saturable kinetics (Fig. 3A). The apparent affinity of each lipoprotein for LTP was nearly the same (20–25 nm). At saturating concentrations of LTP, VLDL bound 1.5 molecules of LTP per VLDL, a value that was 12-fold higher than the maximum binding calculated for LDL and 70-fold higher than that for HDL.

As shown in Fig. 3B, similar results were obtained for the binding of highly purified LTP to lipoprotein-Sepharose. As
beled HDL (40 pg of cholesterol) as donor and a constant VLDL eluates from VLDL-Sepharose columns were assayed using radiola-

used) employed in Fig.

the same (-55 nM)-a value that is 2-fold higher than the

constant LDL acceptor concentration of 320 pg of cholesterol and

using radiolabeled HDL (112 pg of cholesterol) as donor and a

constant VLDL, LDL, and HDL for highly purified LTP were nearly

comparison of the extent of LTP binding in these two studies

can be made directly. However, when partially purified

LTP (260 pg of protein) in Tris/NaCl buffer containing 0.05% BSA plus

the indicated amount of HDL, LDL, or VLDL (1.5 ml total volume)

were premixed for 15 min and then applied to 0.5-ml columns (0.55

x 2 cm) of HDL-Sepharose (11.1 nmol, 350 µg of cholesterol), LDL-

Sepharose (2.4 nmol, 2.3 mg of cholesterol), or VLDL-Sepharose (0.13

nmol, 435 µg of cholesterol) equilibrated in the same buffer and eluted

at 15-20 ml/h. After sample application, each column was subse-

quently rinsed twice with 0.5 ml of column buffer and the total eluate

was assayed for lipid transfer activity. Eluates from HDL-Sepharose columns (i.e. eluates containing soluble HDL) were assayed using radiolabeled HDL (96 µg of cholesterol) as donor and a constant HDL acceptor concentration of 120 µg of cholesterol (i.e. compensated for HDL in eluates). Eluates from LDL-Sepharose columns were assayed using radiolabeled HDL (112 µg of cholesterol) as donor and a constant LDL acceptor concentration of 320 µg of cholesterol and eluates from VLDL-Sepharose columns were assayed using radiolabeled HDL (40 µg of cholesterol) as donor and a constant VLDL acceptor concentration of 87 µg of cholesterol. Assays were for 4.5 h. Data are the average of duplicate determinations and are presented as the percentage of LTP bound relative to that bound in the absence of soluble lipoproteins. LDL- and HDL-Sepharose columns bound 80-82% and VLDL-Sepharose bound 45% of the applied LTP in the absence of added lipoproteins. These results are representative of two similar experiments.

estimated from double reciprocal plots, the apparent affinities of VLDL, LDL, and HDL for highly purified LTP were nearly the same (≈35 nM)—a value that is 2-fold higher than the apparent affinity of these lipoproteins for partially purified LTP (Fig. 3A). Since some of the experimental conditions (i.e. the lipoprotein-Sepharose preparations and the amounts used) employed in Fig. 3, A and B differ, a quantitative comparison of the extent of LTP binding in these two studies cannot be made directly. However, when partially purified LTP was assayed under the conditions described in Fig. 3B (data not shown), differences in partially and highly purified LTP were observed. Although the maximum binding of partially purified and highly purified LTP to LDL was nearly the same, VLDL bound 28% less highly purified LTP whereas HDL bound 49% more of the purified protein. Thus, the affinity of plasma lipoproteins for LTP is not greatly affected by the other proteins present in partially purified LTP, but these proteins may alter the extent to which LTP binds to each lipoprotein and, consequently, these proteins may have important functions in vivo.

Two approaches were taken to evaluate a possible correlation between LTP binding to plasma lipoproteins and lipid transfer activity. In the first, the dependence of lipid transfer activity and LTP binding on the amount of LTP added to assays was investigated. The transfer of cholesteryl ester from radiolabeled HDL to unlabeled LDL was assayed under conditions where the molar concentration of HDL in the assay was >60-fold higher than that for LDL. Based on the data in Fig. 3, these assay conditions should result in most of the
Lipid Transfer Protein

FIG. 4. Correlation of LTP binding and lipid transfer activity. Radiolabeled HDL (635 pmol, 20 μg of cholesterol) as donor, LDL (10 pmol, 10 μg of cholesterol) as acceptor, and LTP (23–150 pmol) were incubated under standard assay conditions to access lipid transfer activity as a function of the amount of LTP added. All solutions, exclusive of LDL acceptor, were combined and warmed to 37 °C. The transfer reaction was initiated by addition of prewarmed LDL. Assays containing 23 and 46 pmol of LTP were incubated for 1.5 h; all others were incubated for 0.5 h. Transfer was stopped immediately by placing samples on ice. Samples were then processed according to the standard procedure. Under these conditions, the extent of transfer did not exceed 12% for any sample. All data (○—○) are expressed as % kt where t = 1.5 h and are the average of duplicate determinations. These results are representative of two separate experiments. The data (□—□) describing the binding of LTP to HDL at 25 °C as a function of LTP concentration are the same as those presented in Fig. 3A, except they are presented as LTP bound versus total LTP added.

TABLE III
Disruption of lipid transfer protein binding to HDL-Sepharose by the plasma-derived inhibitor

<table>
<thead>
<tr>
<th>Sample applied to HDL-Sepharose column</th>
<th>% LTP bound</th>
<th>LTP binding prevented or displaced by inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTP alone</td>
<td>94</td>
<td>0</td>
</tr>
<tr>
<td>Inhibitor applied with LTP</td>
<td>56</td>
<td>40</td>
</tr>
<tr>
<td>Inhibitor applied before LTP</td>
<td>40</td>
<td>57</td>
</tr>
<tr>
<td>Inhibitor applied after LTP</td>
<td>54</td>
<td>43</td>
</tr>
</tbody>
</table>

lipoprotein-bound LTP being associated with HDL. Thus, these conditions permitted the direct comparison of the binding assays in which only HDL was present, with lipid transfer activity assays, which contained both HDL (donor) and LDL (acceptor). As seen in Fig. 4, with increasing concentrations of LTP, lipid transfer activity and the amount of LTP bound to HDL rose in parallel; both saturated at about the same LTP concentration.

In the second approach to correlate LTP binding with lipid transfer activity, the mechanism by which the plasma-derived inhibitor protein diminished lipid transfer activity was investigated. LTP was applied to columns of HDL-Sepharose either alone or with partially purified inhibitor protein and the binding of LTP to HDL was measured. Applied alone, 94% of the LTP and >95% of the inhibitor activity bound to the HDL-Sepharose (Table III). The inhibitor significantly decreased the amount of LTP bound; this decrease was evident irrespective of whether the inhibitor was applied with, before, or after the LTP had been applied to the HDL-Sepharose column. A plasma fraction of equal protein content but depleted of inhibitor protein (the phenyl-Sepharose unbound fraction, see Ref. 12) had no effect on LTP-HDL binding. Thus, it appears that the inhibitor competes with LTP for binding to the HDL surfaces, prevents the formation of LTP-HDL complexes, and facilitates their dissociation. This suggests that the inhibitor may suppress lipid transfer activity by disrupting LTP-lipoprotein interactions. To investigate this further, the capacity of various amounts of inhibitor to prevent the formation of LTP-HDL complexes was compared to the ability of equal amounts of inhibitor to decrease lipid transfer activity. As in Fig. 4, this experiment (Fig. 5) measured lipid transfer from radiolabeled HDL to unlabeled LDL under conditions where the molar concentration of HDL was >60-fold higher than that for LDL, i.e. LTP-lipoprotein interactions occurred almost exclusively on HDL. As shown in Fig. 5, a good correlation was observed between the extent of lipid transfer activity inhibition and the percentage of LTP binding dissociated by increasing levels of inhibitor. Collectively, the data in Figs. 4 and 5 strongly support a correlation between lipid transfer activity and the binding of LTP to its lipoprotein substrate.
Lipid Transfer Protein

Since binding studies could not be carried out on LTP in whole plasma, the CM-cellulose fraction of LTP was selected and used for most of the studies presented here. The choice of this fraction of LTP was directly by several factors: this fraction is easily prepared and the lipid transfer activity in these preparations is stable for long periods of time, >90% of the LTP in this fraction is unassociated with other proteins since it shows the same molecular weight by gel filtration as purified LTP (18) and this fraction is depleted of the inhibitor protein which is present in less purified fractions (19). Additionally, data have been presented herein which demonstrates that partially and highly purified LTP are bound by VLDL, LDL, and HDL with similar kinetics. This observation is consistent with other studies (8) which have shown that the mechanism of LTP action, as measured by activation parameters, is the same regardless of whether crude (250-fold purified) or highly purified (28,000-fold) LTP is studied.

The present study has demonstrated that partially purified LTP binds to plasma VLDL, LDL, and HDL. A similar binding capability was observed for LTP in whole plasma and for highly purified LTP. Although previous studies (9) suggest that LTP binds avidly and irreversibly to HDL, the studies described here demonstrate that LTP binding to VLDL, LDL, as well as HDL, is dynamic, with bound LTP undergoing rapid dissociation and reassociation between lipoproteins. Equilibrium binding studies indicated that VLDL, LDL, and HDL have approximately the same affinity for LTP, but markedly different binding capacities at saturating levels of LTP. The binding event, as measured under these conditions, was shown to be an integral part of the transfer reaction; conditions which increased binding also increased lipid transfer activity, whereas disrupting the binding event was concomitant with diminished lipid transfer activity.

The observed binding of LTP to all lipoproteins is in contrast to the results of other studies which used gel filtration (9) and ultracentrifugation techniques (28) to evaluate binding. Due to the lability of VLDL-LTP and LDL-LTP complexes observed in the present study, it seems likely that these complexes would dissociate early in the chromatographic procedures used by others and would elute as free LTP. Also, in experiments not shown, the association of LTP with isolated LDL and VLDL was found to be completely disrupted by the single step ultracentrifugation procedure used by Groener et al. (28); 80% of HDL-LTP complexes were also dissociated by this technique. Thus, it appears that previous methods were not suitable to resolve the labile binding of LTP to VLDL and LDL.

Whether LTP binds to lipoproteins does not appear to be dependent on lipoprotein size (i.e. surface curvature or the packing of phospholipid molecules on the surface (15)) or apoprotein composition, since LTP readily interacts with VLDL, LDL, and HDL; however, these factors may affect the extent of LTP binding. Additionally, these characteristics may be important in defining the stability of LTP-lipoprotein complexes in non-equilibrium conditions and may explain why LTP-HDL complexes are long lived, whereas LTP-VLDL and LTP-LDL complexes rapidly dissociate. For example, Pattnaik and Zilversmit (9) demonstrated that the binding of LTP to HDL was primarily electrostatic via interaction with surface phospholipid phosphate groups and that the stability of LTP-lipoprotein complexes under non-equilibrium conditions was increased when the density of negative charges on the lipoprotein was increased by any of several methods.

The finding that VLDL, LDL, and HDL have equal affinities for binding LTP is in contrast to conclusions reached by kinetic analysis of the transfer reaction’s dependence on donor and acceptor lipoprotein concentrations (7). The mathematical model based on the kinetic data included a "δ" parameter—a complex term reflecting both the relative avidity of a lipoprotein for LTP and the relative number of esterified cholesterol molecules per lipoprotein. Based on lipoprotein composition data (15), the contribution of the lipoprotein-esterified cholesterol content to the δ value can be estimated, assuming all cholesteryl esters to be available for transfer. The residual δ values, i.e. terms presumably reflecting the relative avidity of a lipoprotein for LTP, are 1:1:12.9 for HDL, LDL, and VLDL, respectively. Thus, the mathematical model predicts equal avidity of LDL and HDL for LTP, consistent with the finding here that LDL and HDL have the same apparent K₅ for LTP. However, the equilibrium binding data presented here are not in agreement with the model’s prediction for VLDL. One possible explanation for this discrepancy may be that the studies here used native VLDL, whereas the studies of Barter et al. (7) used VLDL isolated from plasma incubated at 37 °C for 24 h, conditions known to alter greatly the lipid and apoprotein composition of VLDL (6). It is presently unknown how these changes may affect LTP binding.

On a molecular basis, VLDL, LDL, and HDL differed markedly (up to 70-fold) in their maximum binding capacity for LTP. At saturating levels of partially or highly purified LTP, LDL, and HDL appeared to bind <1 molecule of LTP per lipoprotein molecule, whereas VLDL bound ≈1.5 (Fig. 3). These results suggest that LDL and HDL, and perhaps VLDL, are heterogeneous with respect to LTP binding and that a small subpopulation in each lipoprotein fraction may be responsible for the observed binding of LTP. This possibility is presently under investigation. Alternatively, the binding of <1 molecule of LTP per molecule of LDL or HDL may be due to steric hindrance and “equilibrium effects” (29) which have been shown to dramatically decrease the capacity of ligands covalently linked to supports. Hence, the observed binding capacities of lipoproteins for LTP should be considered minimum values. Interestingly, if these data are assumed to reflect relative binding capacities, then the binding capacities of VLDL, LDL, and HDL for LTP, which differed by 70-fold when calculated on a molecular basis, were less than 3-fold different when expressed as the amount of LTP bound per unit of lipoprotein surface area. This is consistent with the findings of Tajima et al. (30) that the maximum binding levels of apolipoproteins A-I, A-II, C-II, and C-III₄ to both large and small phosphatidylycholine-triglyceride particles are similar when the data are expressed as amino acids bound per phospholipid molecule.

The data presented in Fig. 3 can be extrapolated to estimate the minimum binding of LTP that occurs at physiological concentrations of lipoproteins. For this extrapolation, the plasma concentrations of VLDL, LDL, and HDL were taken as 0.067, 1.87, and 12.8 μM, respectively (15, 16, 31), the
plasma concentration of LTP was estimated to be 60 nM (3) and it was assumed that LTP binding is proportional to the lipoprotein concentration when the unbound (free) LTP concentration is kept constant. From these calculations, it is apparent that the minimum binding capacity of lipoproteins for LTP exceeds the plasma concentration of LTP by at least 10-fold. HDL alone has a binding capacity that is at least several times the plasma LTP concentration and could, therefore, bind essentially all the LTP dissociated from LDL and VLDL during the gel filtration of plasma (9)—thus giving the appearance that LTP associates only with HDL. Thus physiologically, lipoproteins are not saturated with LTP and, in fact, only a small part of the total potential is utilized. Changes in HDL, LDL, and/or VLDL pool size, therefore, may be accompanied by shifts in LTP distribution among plasma lipoproteins; this may explain, in part, the abnormal lipid transfer activities noted in the plasma of patients with abnormal lipoprotein profiles (32). From these calculations, it is estimated that about half of the plasma LTP is bound to HDL, with most of the remaining LTP bound to the VLDL-LDL fraction. The plasma concentration of unbound (free) LTP is calculated to be only 7% of the total plasma LTP level or about 4 nM—a concentration that is only 1/3 to 1/6 of the apparent \( K_d \) for LTP binding to lipoproteins. This low level of unbound LTP is consistent with gel filtration studies of whole plasma which show that little lipid transfer activity elutes from the column in the unbound form (9). The calculated distribution of LTP among the various plasma lipoproteins is tentatively supported by preliminary studies which have used anti-Apo-B-Sepharose to partially deplete plasma of VLDL-LDL; approximately 40-60% of plasma LTP was determined to be associated with this lipoprotein fraction.

Two lines of evidence have been presented which suggest that the binding of LTP to the lipoprotein surface is a requisite for transfer activity. These are: 1) increased LTP-donor lipoprotein interactions were paralleled by increments in lipid transfer activity and 2) disruption of LTP-donor lipoprotein interactions by the inhibitor protein was concomitant with a similar decrement in lipid transfer activity. Additionally, Ihm et al. (2) have demonstrated that transfer activity is reduced at low pH or at pH 7.4 in the presence of calcium, conditions known to cause LTP-HDL complex dissociation (9). It is interesting to speculate that changes in lipoprotein composition may affect LTP binding and, therefore, the extent that a lipoprotein participates in the transfer process. For example, Fielding et al. (33) have shown that the abnormal lipoproteins of diabetic patients participate poorly in transfer reactions and Tall et al. (34) have demonstrated that lipoprotein lipase-treated VLDL is a much better substrate for LTP than native VLDL. Additionally, we have found that reconstituted HDL lacking sphingomyelin was a 2-fold better substrate for LTP than reconstituted HDL containing 20 mol % sphingomyelin, and Sweeney and Jonas (35) have reported that plasma phospholipid transfer activity is considerably affected by the amount of free cholesterol and sphingomyelin in donor membranes. Whether LTP binding parallels the apparent participation of these lipoproteins in the transfer reaction is yet to be determined.

\[3\] R. E. Morton and A. K. Osborne, unpublished observation.

In conclusion, the results of this study demonstrate for the first time that the plasma-derived LTP binds reversibly to all lipoproteins (VLDL, LDL, and HDL). The binding event was characterized by high affinity, saturable kinetics; all lipoproteins demonstrated approximately the same affinity for LTP. This study also provides evidence which strongly suggests that an essential step in the lipid transfer process is the physical association of LTP with its lipoprotein substrate. Further studies are required to determine how aberrant lipoprotein compositions, such as those noted in various hyperlipemias, might affect the formation of LTP-lipoprotein complexes and, ultimately, the participation of those lipoproteins in the lipid transfer reaction.

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REFERENCES