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SIMULTANEOUS TRANSFER OF CHOLESTERYL ESTER AND PHOSPHOLIPID BY PROTEIN(S) ISOLATED FROM HUMAN LIPOPROTEIN-FREE PLASMA

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SUMMARY

Protein(s) catalyzing the transfer of [^{3}H]cholesteryl ester and [^{14}C]-phosphatidylcholine from high density lipoproteins to low density lipoproteins have been purified 4829-fold from human plasma by chromatography of the d > 1.21 g/ml infranatant fraction of human plasma on phenyl-Sepharose, CM-cellulose, concanavalin A-Sepharose, and finally by isoelectric focussing. At each step of the purification, both transfer activities coelute. The purified protein(s), molecular weight 150,000, transfer cholesteryl esters and phosphatidylcholine with a 1:1 stoichiometry and at equal rates of flux. Rat plasma contains a protein(s) which facilitates the transfer of phosphatidylcholine as effectively as human plasma. However, the rat plasma protein(s) does not facilitate the transfer of cholesteryl esters. These results suggest that human plasma contains one or more proteins which transfer both lipids, possibly as a 1:1 complex, whereas rat plasma lacks the cholesteryl ester transfer protein.

INTRODUCTION

Although it was reported in 1965 (1) that cholesteryl esters (CE)[1] transfer from HDL to VLDL, only recently have the proteins which facilitate this transfer been isolated and characterized. Zilversmit et al. (2) and Pattnaik et al. (3,4) isolated a glycoprotein from the density >1.25 g/ml infranatant which catalyzes the exchange of CE between all of the plasma lipoproteins. The exchange protein has a molecular weight of approximately 80,000 and an isoelectric point of 5. Sniderman et al. (5) also demonstrated that a protein in the d > 1.25 g/ml plasma fraction facilitates exchange of CE from HDL to LDL.

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[1]Abbreviations: VLDL, very low density lipoproteins (d 1.006 g/ml); LDL, low density lipoproteins (d 1.02-1.05 g/ml); HDL, high density lipoproteins (d 1.063-1.21 g/ml); CE, cholesteryl esters; PC, phosphatidylcholine; PL, phospholipids; Tg, triglyceride; LCAT lecithin:cholesterol acyl transferase; conA, concanavalin A.

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In contrast to the facilitated exchange process described above, Chajek and Fielding (6) isolated a protein which facilitates the net transfer of CE from HDL to LDL or VLDL and the back transport of Tg to HDL. This protein has a molecular weight of 35,000 and, based on its properties, Chajek and Fielding (6) suggest that it may be apoD (7,8). Barter et al. (9) purified a serum transfer protein which mediates the bidirectional transfer of Tg between lipoprotein classes; the protein is a glycoprotein with an isoelectric point of 9.0.

While it is evident that detailed knowledge of the proteins which facilitate the transfer of CE and Tg is still lacking, it seemed appropriate to reexamine the lipid specificity of the plasma phospholipid exchange protein (10). This protein is isolated from the d > 1.21 infranatant fraction, has a molecular weight >100,000 and facilitates the bidirectional transfer of PC. The phospholipid transfer protein was purified 4829-fold and characterized, and its ability to facilitate transfer of PC and CE was assessed.

MATERIALS AND METHODS

Materials. 1-Stearoyl-2-oleoyl phosphatidylcholine, purchased from Applied Science Laboratories, was determined to be pure by thin-layer chromatography on silica gel (chloroform:methanol:water, 65:25:4). Egg yolk [choline-methyl-^{14}C]phosphatidylcholine (50 mCi/mmol) and [7-^{3}H(N)]cholesterol (11 mCi/mmol) were purchased from New England Nuclear. Fatty acid-free bovine serum albumin was obtained from Sigma Chemical Company. Phenyl-Sepharose CL-4B, conA-Sepharose 4B and Sephadex G200 were purchased from Pharmacia Fine Chemicals; CM-52 was obtained from Whatman. Amphilites (pH 4.0-6.0) were obtained from LKB Instruments.

Isolation of human plasma lipoproteins and d > 1.21 infranatant. Lipoproteins were isolated from the freshly-collected plasma of normolipemic fasted human volunteers by sequential ultracentrifugal flotation in salt solutions of KBr. Each lipoprotein class was refloated at its respective density and finally dialyzed against 0.9% NaCl, 0.001 M EDTA, 0.001% sodium azide, pH 7.4. The chemical composition of the lipoproteins was determined by the methods of Hui and Harmony (11). The purity of the lipoprotein fractions was assessed by electrophoresis on agarose (1%, pH 8.6) and by immunodiffusion using antibodies raised against LDL, HDL and apoB. Following the removal of HDL, the d > 1.21 infranatant, excluding the clear zone immediately below the lipoprotein layer, was employed as the source of the transfer protein.

Description of assay. Double-labeled HDL containing [^{14}C]PC and [^{3}H]CE were prepared as follows: HDL were labeled with [^{3}H]cholesterol as described by Chung et al. (12). The [^{3}H]cholesterol in HDL was then esterified with partially purified LCAT (13). After incubating with a 10-fold excess of LDL to remove unesterified cholesterol, the HDL were reisolated. [^{14}C]PC was incorporated into [^{3}H]CE-HDL by using [^{14}C]PC-labeled LDL and purified bovine

liver PC exchange protein (14,15). [^{14}C]PC-labeled LDL was prepared by incubating sonicated vesicles of 1-stearoyl-2-oleoyl phosphatidylcholine containing egg [^{14}C]PC, LDL and liver PC-exchange protein as described previously (15). Each assay contained [^{3}H]CE-[^{14}C]PC-labeled HDL and unlabeled LDL in 1.0 ml of 0.9% NaCl, 10 mM Tris-Cl, 1 mM EGTA, pH 7.4. Incubations were performed at 37°C either in the presence or in the absence of transfer protein(s). After a specified period of incubation, the density of incubation mixture was raised to 1.07 g/ml by addition of solid KBr. The d 1.07 solution was then layered with d 1.063 KBr solution, and the mixture was centrifuged for 19 h at 48,000 rpm in a Beckman 50 Ti rotor. LDL which formed a thin surface film and HDL which remained in the bottom of the tube were collected. An aliquot (usually 0.5 ml) of each lipoprotein solution was counted in 10 ml Aquasol (New England Nuclear). The phospholipid or cholesterol content of each lipoprotein solution was determined to calculate the percent recovery of lipoproteins.

Calculations. The maximal theoretical transfer of [^{14}C]PC from HDL to LDL was calculated from the formula [Y/(X+Y)]a cpm, where X and Y are the total HDL PC and LDL PC pools, respectively, and a is the HDL [^{14}C]PC radioactivity. The maximal expected transfer of [^{3}H]CE was calculated in the same manner. The extrapolated, equilibrium value for PC or CE transfer was obtained by a double-reciprocal plot of the counts transferred from HDL to LDL *versus* the incubation time.

The rate of exchange of PC and CE was calculated assuming that there is exchange only, with no net transfer of mass, and that each lipid exists in a single homogeneous pool, all of which is available for exchange. The flux rate was determined using the formula which was derived by Barter and Jones (16).

$$S_H(t) = [S_H(0) - S_{EQ}] \exp - F \left(\frac{M_L + M_H}{M_L \cdot M_H} \right) t + S_{EQ}$$

where $S_H(t)$, $S_H(0)$ and S_{EQ} denote the specific activity of CE or PC (CPM/nmol) in HDL at time t(h), at zero time and at equilibratium, respectively; M_L and M_H denote the CE or PC pool sizes (nmol) in LDL and HDL, respectively, and F denotes the rate of exchange (nmol/h) between LDL and HDL.

RESULTS

Purification procedures of the transfer protein(s) are essentially those for isolation of the CE exchange protein described by others (3,6,9). The plasma d > 1.21 infranatant is applied directly to phenyl-Sepharose, and the majority of the protein is eluted with 0.15 M NaCl (Fig. 1). Both CE and PC transfer activities are retained by the resin and elute with water. In the second step, the fractions containing transfer activity are combined and chromatographed on CM-cellulose (data not shown). The elution profile is nearly identical to that reported by Pattniak et al. (3); both CE and PC transfer activities coelute from CM-cellulose. The active fractions from CM-cellulose are chromatographed on conA-Sepharose, and the result is shown in Fig. 2; CE

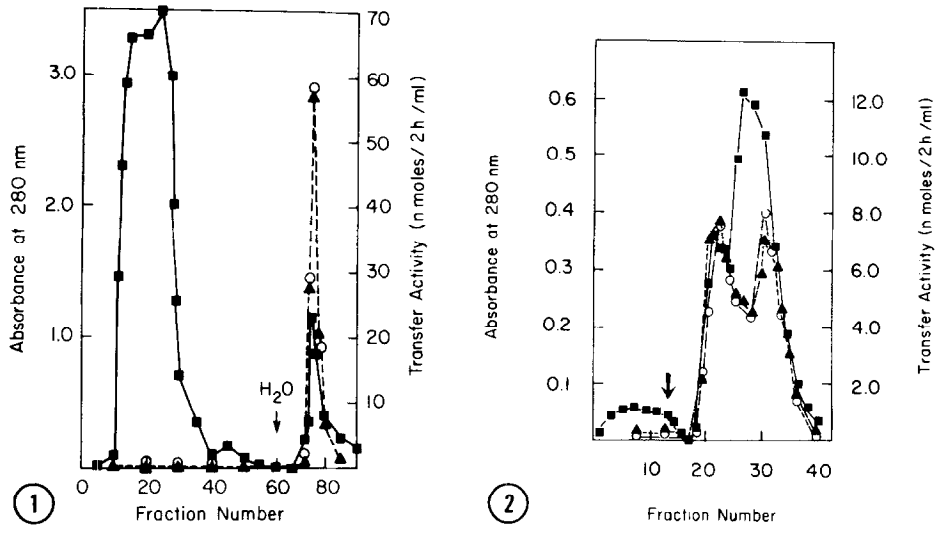
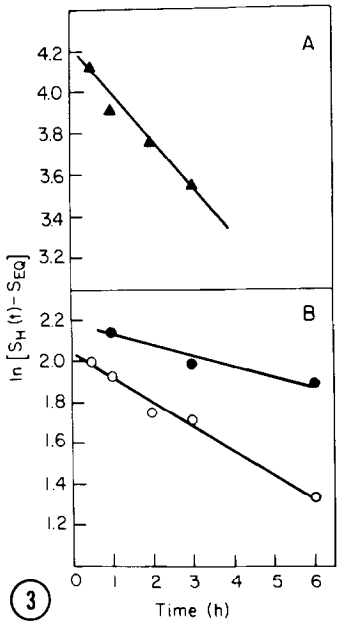


FIGURE 1. Chromatography of transfer protein(s) on phenyl-Sepharose. The column (2.5 x 30 cm) was equilibrated with 150 mM NaCl, 0.02% sodium azide, pH 7.4. The sample (5460 mg protein), corresponding to the infranatant fraction of 500 ml of normal plasma at d > 1.21 (see Results for details), was applied to the column and eluted with the equilibration buffer. At the arrow, water was applied to the column. The flow rate was 180 ml/h and 8 ml fractions were collected. Absorbance at 280 nm (□); CE transfer (▲); PC transfer (o).

FIGURE 2. Chromatography of transfer protein obtained from CM-cellulose on conA-Sepharose. The column (1.6 x 30 cm) was equilibrated with 10 mM Tris-HCl, pH 7.4, 0.1 mM $CaCl_2$. The sample (420 mg protein) corresponding to the active pool from CM-cellulose, was applied and eluted (12 ml/h) with the starting buffer. At the arrow, the buffer was changed to 150 mM α-methyl mannoside; 2.8 ml fractions were collected. Absorbance at 280 nm (□); CE transfer (▲); PC transfer (o).

and PC transfer activities are retained by the affinity resin, and both activities elute with 150 mM α-methyl mannoside. The conA-Sepharose and the CM-cellulose steps can be reversed. The molecular weight of the active preparation as determined on Sephadex G200 is ∿150,000. Isoelectric focussing between pH 4.0-6.0 of the active fraction from conA-Sepharose produces a single active band, isoelectric point of 5.2, consisting of both CE and PC transfer activities. The overall purification of CE and PC transfer activities from plasma is 4829-fold.

The exchange of CE and PC between HDL and LDL was assessed as a function of time, and the flux rates were determined from Fig. 3. In the absence of transfer protein(s), there is essentially no exchange of CE in 6 h. With transfer protein(s), the flux rate is 17.5 nmol CE per h. In contrast, there



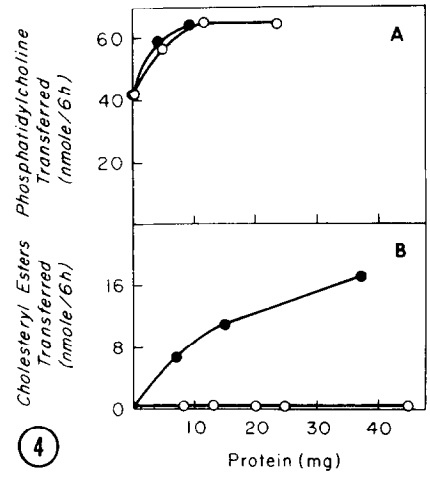


FIGURE 3. Exchange of lipids between HDL and LDL facilitated by the transfer protein(s) from human plasma: (A) CE and (B) PC. [^{3}H]CE, [^{14}C]PC-HDL (80 nmol CE, 280 nmol PC) and LDL (1538 nmol CE, 1867 nmol PC) were incubated in 10 mM Tris-Cl (pH 7.4) containing 0.9% NaCl and 1 mM EGTA at 37°C in the presence (▲, o) or absence (●) of transfer protein (0.4 mg). The data are presented as $\ln[S_H(t) - S_{EQ}]$ vs. time, and the flux rate in nmol/h is the slope divided by $[-(M_L + M_H)/(M_L \cdot M_H)]$, as described in Methods.

FIGURE 4. Exchange of lipids between HDL and LDL facilitated by the transfer protein(s): (A) PC and (B) CE. [^{3}H]CE,[^{14}C]PC-HDL (110 nmol CE, 380 nmol PC) and LDL (1430 nmol CE, 1750 nmol PC) were incubated in 10 mM Tris-Cl (pH 7.4) containing 0.9% NaCl and 1 mM EGTA at 37°C for 6 h in the absence or presence of human transfer protein(s) from human (●) or rat (o) plasma.

is a significant amount of non-facilitated transfer of PC (F, 12.1 nmol/h). However, the protein-catalyzed rate of PC flux is 29.2 nmol/h, indicating that facilitated PC transfer proceeds at a rate of 17.1 nmol/h. At all time points, the stoichiometry of facilitated CE and PC transfer is 1:1; at equilibrium 67 nmol of CE and 73 nmol PC have exchanged. These values represent 98% and 97%, respectively, of the calculated maximum extent of exchange. Chemical analysis of HDL and LDL before and after reaction indicate that net transfer of CE and PC does not occur. Furthermore, thin layer chromatography of LDL lipids after transfer showed that all of the [^{14}C]PC was as phosphatidylcholine.

The data shown above suggest that CE and PC transfer are coupled processes Since rat plasma has much less CE transfer protein than human plasma (17), the transfer properties of the rat and human protein(s) were compared. The rat

plasma preparation chromatographs on phenyl-Sepharose in a manner identical to that illustrated in Fig. 1. Both human and rat transfer protein(s) facilitate the transfer of PC as is indicated in Fig. 4. Furthermore, the rates of PC transfer are qualitatively the same. In contrast, the rat protein(s) does not facilitate transfer of CE. Rat plasma proteins, isolated from the fractions retained on phenyl-Sepharose, do not inhibit CE transfer which is facilitated by the transfer protein isolated from human plasma (data not shown). These data suggest that rat plasma does not contain an inhibitor of CE transfer.

DISCUSSION

The protein(s) from human plasma which facilitate exchange of phospholipid and cholesteryl esters between lipoproteins copurify through four steps. The active material, molecular weight ∿150,000, is amphipathic: it is sufficiently hydrophobic to adsorb to phenyl-Sepharose and it contains carbohydrate moieties which are bound by conA. The most purified preparation of transfer protein enhances exchange of equimolar amounts of PC and CE per unit time. Preliminary data indicate that the active preparation also contains LCAT: it catalyzes the esterification of cholesterol as determined in an assay system of [^{3}H]cholesterol-HDL and albumin (ratio 3:5, protein wt/wt). The data on the transfer protein(s) may be satisfactorily accounted for if: (a) the same protein facilitates transfer of a 1:1 (mol/mol) PC:CE complex; (b) the preparation consists of at least two proteins present in equimolar amounts, one of which mediates exchange of PC and one of which facilitates transfer of CE; or (c) the active unit is a small lipoprotein complex with stoichiometric amounts of PC and CE, such as is the case of HDL, and at least one protein constituent plus LCAT. Since the lipid content of the active preparation has not been determined, the experimental data support alternative (b). The transfer of CE clearly requires a transfer protein since no CE is exchanged between HDL and LDL. Furthermore, the partially purified preparation from rat plasma does not mediate CE exchange The situation is not so well-defined for PC exchange; unfacilitated PC transfer is relatively facile. It is possible that the PC transfer protein is an apo-

protein of HDL. The fact that the partially purified rat preparation facilitates PC transfer uncoupled from CE transfer, though not unequivocal, supports the existence of distinct PC and CE transfer proteins. Taken together, the data suggest that a small complex, present in low steady-state concentration, functions in lipid homeostasis. This complex may represent the catalytic unit responsible for formation and redistribution of plasma cholesteryl esters and for removal of cholesterol from lipoproteins and tissues.

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