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Isolation and characterization of a human serum cholesteryl ester transfer protein

(cholesteryl ester–triglyceride exchange/lipoprotein/transport protein)

T. CHAJEK AND C. J. FIELDING

Cardiovascular Research Institute and Department of Physiology, University of California, San Francisco, California 94143

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ABSTRACT **Human plasma has been shown to contain an apolipoprotein that mediates the transport of cholesteryl ester from high density lipoprotein (HDL) to very low density lipoprotein (VLDL) or low density lipoprotein (LDL). This activity, confined to the density >1.063 g/ml interval, has been isolated from HDL and appears as a single migrating species by anionic or sodium dodecyl sulfate/polyacrylamide gel electrophoresis. It is unreactive to antibodies to the major HDL apolipoproteins. Antibodies prepared against this factor and immobilized on Sepharose remove the capacity of HDL and density > 1.21 g/ml fractions as well as whole plasma to transport cholesteryl ester from HDL. The system shows saturation kinetics with respect to plasma LDL and VLDL concentrations, and transport of cholesteryl ester was associated with reciprocal and equimolar back-transport of triglyceride from VLDL and LDL to the HDL fraction. The possible relationship of this apoprotein to apoprotein D is discussed.**

In human plasma, cholesterol esterification occurs predominately via the lecithin:cholesterol acyltransferase (LCATase; EC 2.3.1.43) reaction. Indeed an acyl-CoA:cholesterol O-acyltransferase could not be detected in human liver (1), and familial LCATase deficiency is associated with the almost complete absence of cholesteryl ester from plasma liporoteins (2). After incubation of lipoproteins from LCAT-deficient subjects with this enzyme, all lipoprotein fractions were found to contain significant levels of cholesteryl ester, even though low density lipoproteins (LDL) and very low density lipoproteins (VLDL) were not significant substrates for the enzyme (3). It therefore has seemed likely that cholesteryl ester, formed by the LCATase reaction from high density lipoprotein (HDL) substrates, is transferred to nonsubstrate lipoproteins by a transport factor whose nature has not been ascertained. It was earlier shown that, when human plasma was incubated at 37°, there was an increase in VLDL cholesteryl ester that was associated with an increased triglyceride content in HDL (4, 5). A cholesteryl ester exchange protein has been identified in the plasma of cholesterol-fed rabbits and is in the density > 1.21 g/ml density fraction (6) although net transport of cholesteryl ester in this case was not shown. Finally, increased cholesteryl ester synthesis by LCATase in human plasma has been shown during alimentary lipemia (7), possibly due to the release of a cholesteryl ester-mediated inhibition of LCATase activity (8). The identity of such a cholesteryl ester transport protein is of considerable potential interest because of its central role in lipoprotein lipid homeostasis. The present research describes the isolation and partial characterization of such a transport protein from human serum.

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METHODS

Preparation of Plasma Lipoproteins. Plasma was taken from blood withdrawn into 0.38% (wt/vol) citrate from healthy volunteers. The major lipoprotein classes (VLDL, $\rho < 1.006$ g/ml; LDL, $1.019 < \rho < 1.063$ g/ml; HDL, $1.063 < \rho < 1.21$ g/ml) were prepared by ultracentrifugal flotation in the presence of 0.1% disodium EDTA (pH 7.4) by using the Beckman L3-40 or L2-65 ultracentrifuges and the 40.3 rotor, with 2-ml adaptor inserts in some experiments (9). Density was adjusted with solid KBr. Lipoproteins labeled in the cholesterol moiety were prepared from plasma preincubated with [*1,2*-^{3}H]cholesterol or [*4*-^{14}C]cholesterol (New England Nuclear, Boston, MA), specific activity 43.9 and 5.36 mCi/mmol, respectively. Typically 100 μCi of [^{3}H]cholesterol was dissolved in 0.25 ml of ethanol and the solution was injected, with stirring, into 10 ml of plasma. The plasma was incubated for 60 min at 37° in the presence of 0.28 mM 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) to inhibit LCAT activity (10). Thereafter, 2 ml of 0.1 M 2-mercaptoethanol was added to reverse the inhibition, and labeled cholesteryl ester was synthesized with endogenous LCATase activity in the course of a further 60-min incubation at 37°. The specific activity of [^{3}H]cholesteryl ester labeled by this procedure was 3042 ± 124 dpm/μg (mean ± SD, six preparations). Irreversible inhibition of LCAT in further incubation was achieved, if required, with 0.1 mM diethyl *p*-nitrophenyl phosphate (E600) (11) (K & K Laboratories, Plainview, NY). Lipoprotein triglyceride was labeled with glyceryl tri-[*9,10*-^{3}H]oleate (New England Nuclear, 490 mCi/mmol) with dimethyl sulfoxide as carrier.* The clear solution of 100 μCi of lipid in pH 7.4 sodium phosphate buffer was incubated with 35 mg of lipoprotein lipid, and the lipoproteins were reisolated within the original density limits. Recoveries were 28% for radioactivity and 53% for triglyceride in typical experiments. The specific activity of lipoprotein triglyceride was 3391 dpm/μg. Lipoprotein apoproteins A-1 and A-2 were prepared by molecular sieve and DEAE-cellulose chromatography (12) from human serum HDL delipidated with ethanol/diethyl ether at −20°.

Preparation of Agarose-Bound Lipoproteins. Labeled lipoproteins were coupled to CNBr-activated Sepharose 4B gel (Pharmacia, Uppsala, Sweden) at a ratio of 10 mg of lipoprotein protein per g of dry gel. Antibodies to lipoprotein apoproteins, HDL, and LDL were prepared by injection of 1.0–5.0 mg of antigen into the foot pads of rabbits with 1 ml of Freund's

Abbreviations: HDL, high density lipoprotein; VLDL, very low density lipoprotein; LDL, low density lipoprotein; LCATase, lecithin:cholesterol acyltransferase (EC 2.3.1.43); DTNB, 5,5′-dithiobis(2-nitrobenzoic acid).

* Lipoproteins labeled in the triglyceride moiety by this method have identical chemical and radiochemical clearance rates in the intact rat (C. J. Fielding and J. P. Renston, unpublished data).

complete adjuvant. After 14 days the same amount of antigen and adjuvant was injected into multiple subdermal sites. Antiserum obtained after a further 14 days was fractionated by ammonium sulfate precipitation and column chromatography on DEAE-cellulose (13). The purified globulin fraction was coupled with CNBr-activated Sepharose. Antibody to arginine-rich protein was the gift of Richard J. Havel.

Analytical Techniques. Lipoprotein protein was determined by the Lowry procedure (14). Lipid extraction (of 0.4-ml lipoprotein samples) was with 1.5 ml of chloroform/methanol, 1:2 (vol/vol). After separation of the phases with chloroform (0.5 ml) and 0.15 M NaCl (0.5 ml) (15), portions of the lower (chloroform) phase were taken for thin-layer chromatography on silica gel layers (Merck, Darmstadt, Germany) developed in hexane/diethyl ether/acetic acid, 83:16:1 (vol/vol). The cholesteryl ester (R_F 0.9–0.95) and in some cases triglyceride (R_F 0.4–0.5) bands were extracted with 5 ml of chloroform/methanol, 1:1 (vol/vol), and then analyzed for radioactivity, cholesterol content, and triglyceride (16, 17). Recovery of cholesteryl ester and triglyceride through the entire procedure was 90–95%. Radioactivity was determined by using a Searle Mark V liquid scintillation counter with [^{3}H]toluene as internal standard. Anionic polyacrylamide gel electrophoresis was according to Davis (18). Migrating protein bands were visualized with 0.02% Coomassie blue in methanol/water/acetic acid, 45:45:10 (vol/vol). Polyacrylamide gel electrophoresis in the presence of 0.1% sodium dodecyl sulfate and 1% 2-mercaptoethanol was according to Weber and Osborn (19). Double immunodiffusion was in plates of 1% agarose in 5 mM barbital buffer (pH 7.4).

RESULTS

Net Transport of Cholesteryl Ester between Plasma Lipoproteins. When plasma was incubated at 37° there was a net increase in VLDL and LDL cholesteryl ester as determined by ultracentrifugal flotation after incubation (Fig. 1). When transport of cholesteryl ester to VLDL or LDL was determined both chemically and radiochemically with the same plasma samples, identical transport rates were obtained. Net transport of cholesteryl ester was linear for at least 3 hr. When DTNB (1.4 mM) was included in the incubation mixture, LCATase activity (assayed chemically in terms of total increase in cholesteryl ester) was 90% inhibited, and the transfer of preformed cholesteryl ester from HDL to LDL and VLDL (which was unchanged) was associated with a corresponding decrease in HDL cholesteryl ester. Transport rates in five human subjects were well correlated with the level of lipoprotein cholesteryl ester in VLDL and LDL (correlation coefficient $r = 0.786$ for VLDL and 0.765 for LDL). Absolute transport rates were 8.1–27.7 nmol of cholesteryl ester per hr per ml of plasma to VLDL and 51.6–118.5 nmol to LDL at 37°.

When HDL was incubated with either LDL or VLDL in the absence of LCATase activity (i.e., in the absence of net production of HDL cholesteryl ester), transport to LDL and VLDL was associated with an equal net decrease in HDL cholesteryl ester (Fig. 2). Incubation of HDL with increasing amounts of LDL and VLDL showed saturation kinetics with half-maximal transport rates at 14–16 mg of LDL and 2–2.5 mg of VLDL cholesteryl ester per 100 ml (Fig. 3).

Reciprocal Transport of Lipoprotein Neutral Lipids. To investigate the transport of cholesteryl ester between lipoprotein molecules from the same density fraction, mixed incubations of Sepharose-bound and soluble lipoproteins were carried out. The transfer of labeled cholesteryl ester or triglyceride was determined after the bound and soluble lipoproteins were separated by centrifugation for 5 min at 500 × g. Similar rates of transport of labeled cholesteryl ester from soluble and from Sepharose-bound HDL to VLDL and LDL were obtained. Thus, ultracentrifugal shearing forces were not a significant factor in the transport of cholesteryl ester in these experiments. Cholesteryl ester transport showed considerable specificity.

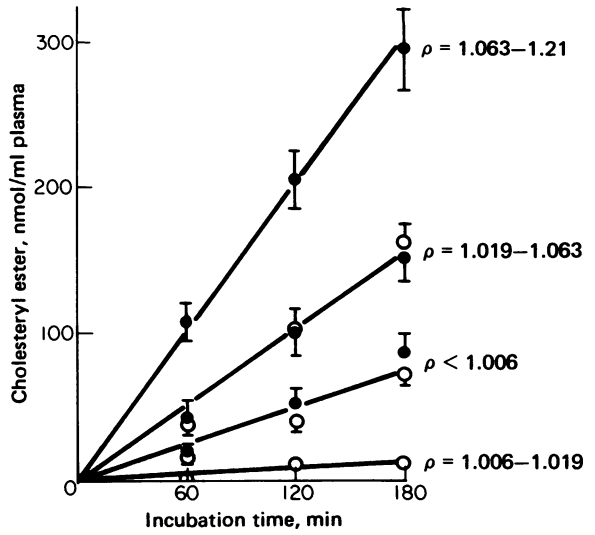

FIG. 1. Chemical (●) and radiochemical (○) determination of cholesteryl ester formation and transport in human plasma. Plasma was labeled with [^{3}H]cholesterol. Thereafter, 2-ml samples were incubated at 37° for an additional 0–180 min. At the end of the incubation period the tubes were cooled in ice and subjected to serial ultracentrifugation at ρ = 1.006, 1.019, 1.063, and 1.21 g/ml at 4°. The radioactivity and cholesteryl ester contents of each density fraction were determined. The rate of transfer of [^{3}H]cholesteryl ester in HDL to LDL or VLDL was calculated relative to the HDL cholesteryl ester specific activity after 60 min of incubations. Results represent means ± SD in triplicate determinations.

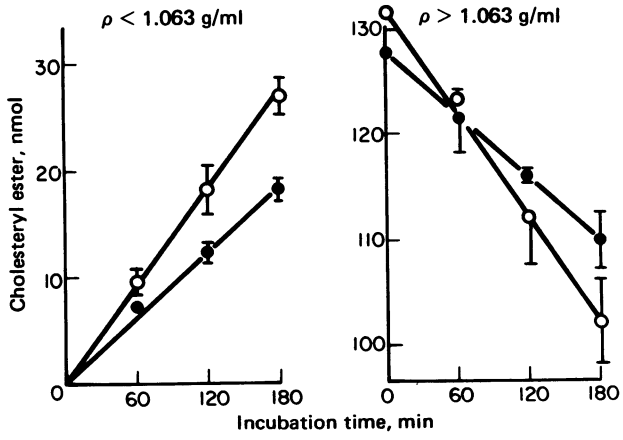

FIG. 2. Transport of cholesteryl ester from HDL to VLDL or LDL. ^{3}H-Labeled HDL cholesteryl ester (50 μg) was incubated with VLDL (100 μg of cholesteryl ester) (●) or LDL (300 μg of cholesteryl ester) (○) in 0.5 ml of reaction medium containing phosphate buffer (0.06 M, pH 7.4) at 37° for up to 180 min. The radioactivity of the cholesteryl ester fraction in density fractions $\rho < 1.063$ and $\rho > 1.063$ g/ml fractions was assayed and the transfer of cholesteryl ester was determined as in Fig. 1. In the absence of added VLDL or LDL, transfer was 0.2–0.4% of added HDL radioactivity. There was no increase in this blank value by incubation at 37°, and no effect of addition of diethyl p-nitrophenyl phosphate (0.1 mM) or DTNB (0.28 mM) on the transfer rate. Results represent means ± SD in triplicate determinations.

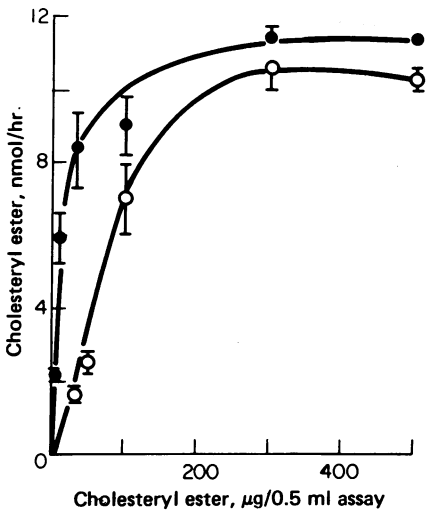

FIG. 3. Rate of transfer of cholesteryl ester as a function of acceptor lipoprotein concentration. Fifty milligrams of ^{3}H-labeled HDL cholesteryl ester was incubated with the indicated concentrations of LDL (○) and VLDL (●) cholesteryl ester in 0.5-ml reaction volumes containing phosphate buffer for 2 hr at 37°. Results represent means ± SD in three to six experiments.

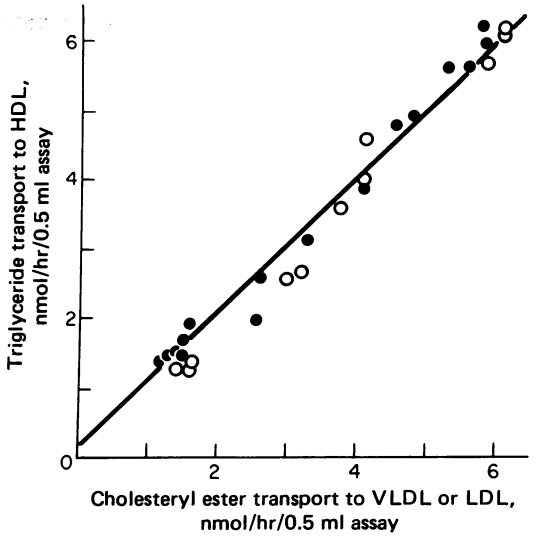

FIG. 4. Reciprocal transport of lipoprotein cholesteryl ester and triglyceride. ^{14}C-Labeled HDL cholesteryl ester (50 μg) was incubated with [^{3}H]triolein-labeled VLDL triglyceride (●) at concentrations 0.135–1.35 mg of triglyceride per ml (19.6–196 μg of VLDL cholesteryl ester) or [^{3}H]triglyceride-labeled LDL triglyceride (○) (4.6–22.9 μg of triglyceride; 38.3–193 μg of cholesteryl ester) for 2 hr at 37°. The reciprocal transport of cholesteryl ester to VLDL or LDL and triglyceride to HDL in each assay was determined as in Fig. 1. $y = 0.955\,x + 0.188$; $r = 0.98$.

Only HDL donated significant amounts of cholesteryl ester to other lipoprotein fractions, and LDL and VLDL were ineffective donors of cholesteryl ester to each other or to HDL (<5% the rate of transport from HDL to other lipoproteins at the same cholesteryl ester concentration). When soluble HDL labeled in the cholesteryl ester, was incubated with VLDL or LDL labeled with [^{3}H]triolein, transport of cholesteryl ester was associated with a reciprocal and equimolar back-transport of triglyceride (Fig. 4). Under the same conditions, transport of triglyceride from HDL (labeled with triolein) to VLDL or LDL was only 5–8% the rate of triglyceride transported from VLDL or LDL to HDL. The mechanism of this process therefore appears to be not the independent exchange of triglyceride and of cholesteryl ester between lipoproteins (because the back transport rates are much lower) but a coupled exchange/transport of HDL cholesteryl ester for VLDL or LDL triglyceride.

Similar experiments were carried out to investigate whether exchanges occurred between different HDL molecules. The

Table 1. Purification of cholesteryl ester transfer protein

Fraction	Protein, mg	Activity, nmol cholesteryl ester hr^{-1}	Specific activity	Fold-purification	Yield, %
Plasma	3096.5	6471	2.1	1.0	100
HDL*	60.4	2272.0	37.6	18.1	35.1
Antibody affinity† step (retained fraction)	3.5	866.6	245	117.8	13.4
Concanavalin A affinity step (retained fraction)	0.5	523.6	1022	491.0	8.1

Fifty-five milliliters of plasma was labeled and the fraction $1.063 < \rho < 1.21$ g/ml was isolated and dialyzed against 0.15 M NaCl. Fifteen milligrams of protein of this fraction was loaded on the antibody column and it was then washed with 0.01 M Tris-HCl, pH 7.4/0.9% NaCl/1 mM EDTA until absorbance of the eluate at 280 μm returned to baseline. The material bound was then eluted with 50 ml of 3 M NaSCN. This was incubated for 30 min with 20 mM 2-mercaptoethanol at 4° and then chromatographed on concanavalin A-Sepharose 4B. The column was washed with 0.15 M NaCl until the conductivity of the eluate returned to baseline, then the retained fraction was eluted with 0.2 M mannoside. Transfer activity at each step was determined by addition of 1–10 μg of purified protein to an assay containing 50 μg of ^{3}H-labeled HDL (depleted of transfer activity by prior treatment through the antibody-Sepharose step) and 300 μg of LDL cholesteryl ester, in the presence of 0.28 mM DTNB. The range of specific activities in these experiments was 745–1272 nmol of cholesteryl ester transferred per hr per mg of protein. When the purified fraction was chromatographed on a column (2.0 × 25 cm) of Sephadex G-150 equilibrated with 0.15 M NaCl (pH 6.8), both protein and transport activity (measured with HDL and LDL as described above) cochromatographed without change in specific activity (933–1005 nmol hr^{-1} mg^{-1} of protein).

* The density fraction $\rho > 1.21$ g/ml contained 2722 mg of protein and 3639 units of transfer activity, accounting for 56.2% of total plasma activity.

† The unretained fraction of the antibody affinity column contained 1047 units of transfer activity, accounting for 16.1% of original total plasma activity.

rate of transfer of cholesteryl ester between Sepharose-bound HDL and soluble HDL was 1.2 ± 0.2 nmol hr^{-1} (mean ± SD in four experiments) under conditions such that the rate of transport to soluble LDL, at the same cholesteryl ester concentrations, was 5.4 ± 0.9 nmol hr^{-1} (mean ± SD in four experiments). The transfer of cholesteryl ester between HDL molecules could have resulted if cholesteryl ester was exchanged for cholesteryl ester between HDL molecules or if cholesteryl ester was transported between both soluble and Sepharose-bound HDL in exchange for triglyceride. When Sepharose-bound HDL labeled with [^{3}H]cholesteryl ester was incubated with soluble HDL labeled with [^{14}C]cholesteryl ester, there was reciprocal and approximately equimolar exchange or transport of these lipids: cholesteryl ester transfer to the Sepharose-bound HDL was 1.7 nmol hr^{-1}, and cholesteryl ester transfer to the soluble HDL was 1.96 nmol hr^{-1}. When HDL labeled with [^{14}C]cholesteryl ester and immobilized on Sepharose was incubated with soluble HDL labeled with [^{3}H]triolein, transfer of equimolar amounts of cholesteryl ester for triglyceride was obtained: 1.53 ± 0.1 nmol hr^{-1} (mean ± SD in three experiments) for cholesteryl ester transferred from the Sepharose-bound to the soluble HDL, and 1.60 ± 0.2 nmol hr^{-1} (mean ± SD in three experiments) for triglyceride transfer from the soluble to the Sepharose-bound HDL. Thus, in the case of HDL also, transport of cholesteryl ester appears to be concomitant with transport of triglyceride. In this case (unlike transport between HDL and LDL or VLDL) it cannot be directly shown that such transport involves the same HDL molecules. Nevertheless, in view of the other similarities between the results it seems most likely that the same mechanism is operative.

Isolation of Plasma Cholesteryl Ester Transport Factor. When the lipoprotein fractions were separated by ultracentrifugal flotation from plasma, most of the transfer activity was associated with the $\rho > 1.21$ g/ml infranatant solution; the bulk of the remainder floated within the HDL density range, and isolation of HDL was associated with a significant purification of transfer activity (Table 1). Fractionation of HDL protein after delipidation with ethanol/ether at −70° was carried out by ion-exchange chromatography on hydroxylapatite. The delipidated protein was dissolved in 1 mM potassium phosphate, pH 8.0/2 M urea at 10 mg of protein per ml. The solute was passed through a column (2 × 40 cm) of hydroxylapatite (Bio-Rad, Richmond, CA) equilibrated with the same buffer. The void volume fraction was collected and shown by anionic polyacrylamide gel electrophoresis to contain two protein bands, one of which comigrated with human apolipoprotein A-1. This fraction was rechromatographed under the same conditions and then showed a single staining band that, by sodium dodecyl sulfate/gel electrophoresis in 1% 2-mercaptoethanol, had an apparent molecular weight of 35,000. This purified protein was inactive in increasing cholesteryl ester transfer when added to HDL in the presence of VLDL or LDL. However, antibody raised against the pure antigen and then immobilized on Sepharose removed transport activity from whole plasma and purified HDL and the $\rho > 1.21$ g/ml infranatant fraction. To compare the effect of this procedure on transport of cholesteryl ester and triglyceride, HDL was passed through the antibody column and transport was measured before and after exposure to antibody. This procedure decreased transfer of cholesteryl ester from HDL to VLDL from 9.0 ± 0.5 to 2.7 ± 0.2 nmol hr^{-1} and transfer of triglyceride from VLDL to HDL from 8.2 ± 1.2 to 2.3 ± 0.5 nmol hr^{-1}.

To purify the transport protein, HDL was prepared from plasma labeled by preincubation with [^{3}H]cholesterol and then passed through a column (1.2 × 16 cm) of Sepharose complexed with antibody raised against the isolated antigen. As shown in Table 1, much of the transfer activity was retained on the column and the eluate had lost 40–50% of its ability to catalyze the transport of HDL to LDL. This remaining activity was decreased an additional 20–30% by repurification through the column. The labeled lipoprotein eluate, depleted of endogenous transport activity, was then used as substrate in the assay transfer of the following purified fractions of transfer protein. The protein retained on the antibody column after elution of HDL was solubilized with 3 M sodium thiocyanate and, after dialysis against three changes of 250 vol of phosphate-buffered saline, was found to retain the bulk of the original activity removed from HDL. By anionic urea/polyacrylamide gel electrophoresis, the fraction was found to contain three protein bands, two of which comigrated with authentic apoproteins A-1 and A-2 from human plasma HDL, and the third of which comigrated with the purified protein isolated as described above from delipidated HDL. Because neither of the apoproteins contains carbohydrate (20, 21), the eluate from antibody column chromatography was further fractionated by chromatography on concanavalin A-Sepharose. The transfer activity was retained on the column and was eluted in good yield with 0.2 M α-methylmannoside. The final product, which was highly active in catalyzing cholesteryl ester transport, contained a single migrating protein species by anionic and sodium dodecyl sulfate/gel electrophoresis (Fig. 5). In the former conditions, the migration rates of the active transport factor and the previously isolated delipidated antigen were identical. Neither preparation showed detectable reactivity with antibodies raised against the A-1, A-2 and arginine-rich proteins of human plasma HDL.

DISCUSSION

It has been previously shown that net transport of cholesteryl ester from HDL to VLDL took place when these lipoproteins, isolated from plasma, were incubated at 37° (4). Although the mechanism was not determined, this pathway has represented

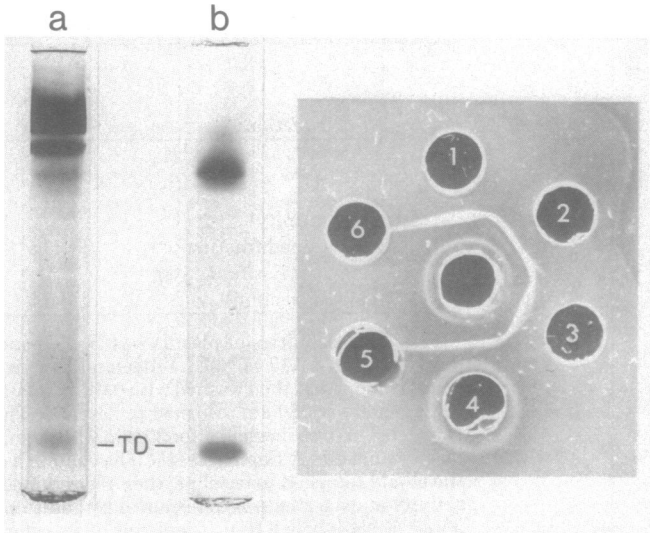

FIG. 5. (*Left*) Polyacrylamide gel patterns of human HDL (lane a) and cholesteryl ester transport protein (lane b). Electrophoresis was in 7% acrylamide in the presence of 8 M urea; 20–50 μg of total protein was applied to the gels. TD, tracking dye. (*Right*) Double-immunodiffusion patterns of cholesteryl ester transport protein. The central well contained antiserum to the transport protein, and the surrounding wells contained the following: 1, delipidated purified transport protein; 2, human HDL; 3, active transport protein purified by antibody and concanavalin A column chromatography; 4, human plasma; 5, apoprotein A-I; 6, apoprotein A-II.

the only reported means by which cholesteryl ester, synthesized by the LCATase reaction with HDL, could be distributed to LDL and VLDL. The present study shows that human plasma contains a specific lipoprotein apoprotein catalyzing the net transport of cholesteryl ester from HDL to VLDL and LDL (Figs. 1 and 2) and, at a low rate, between HDL molecules. This transport is associated with an equimolar back-transport of triglyceride to HDL (Fig. 4). Such transport represents a saturable process for both lipoprotein acceptors of cholesteryl ester; however, in view of the greater plasma concentration of LDL, the rate in whole plasma is 3- to 8-fold greater with LDL. This may not necessarily represent the relative rates *in vivo*. First, newly formed VLDL may contain little or no cholesteryl ester (1, 2) whereas the particles isolated from plasma contain significant proportions, apparently obtained via the transfer process, which may only accept cholesteryl ester at a reduced rate; second, *in vivo* lipolysis of VLDL triglyceride may increase the rate of transport of cholesteryl ester to VLDL.

Although the present study shows an equimolar exchange of triglyceride and cholesteryl ester, it is not yet possible to conclude that such exchange is an obligatory part of cholesteryl ester transport. However, it seems apparent that potentially active transfer pathways exist with both VLDL and LDL as recipients of HDL cholesteryl ester. The ratio of triglyceride and cholesteryl ester molecular volumes (molecular weight/density) is about 1.7 and hence the pathway described would be expected to increase the size of HDL and to decrease that of LDL and VLDL. It may be relevant that, in both genetic and acquired deficiencies of plasma LCAT activity, in which cholesteryl ester is unavailable for transfer, anomalous large and triglyceride-rich LDL are a feature of the serum lipoprotein pattern (2, 22). It seems possible that the normal catabolism of VLDL to LDL requires the presence of both HDL and plasma cholesteryl ester transfer factor. The fatty acid composition of triglyceride is similar in VLDL and HDL (23), which suggests that a significant part of total HDL triglyceride may be derived from the pathway described here.

The purification procedure for the delipidated, inactive transfer protein is similar to that described for preparation of apo D, a minor polypeptide of HDL (24), as is its migration rate in polyacrylamide gel electrophoresis, and the balance of evidence presented here strongly suggests the identity of plasma cholesteryl ester transport factor and apo D. However, several quite different descriptions of the chemical properties of this protein have been reported (24–26), with reported molecular weights (by sodium dodecyl sulfate gel electrophoresis) of between 19,000 and 26,000, and the identity of the apo D fraction as a single polypeptide species remains to be established. The present study shows that a higher molecular weight protein component of HDL prepared as described for apo D can be isolated by affinity chromatography and represents the major or only factor active in the transfer of cholesteryl ester to VLDL and LDL in plasma. The rate of transport identified is comparable to the rate of cholesterol esterification by LCATase *in vitro* and this rate is similar to the rate of total plasma cholesteryl ester formation *in vivo* (27, 28). Because LCATase appears to react specifically with HDL (3), it seems likely that the transport mechanism described here could be a major pathway *in vivo* for disposition of HDL cholesteryl ester.

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