Kinetics of plasma protein-catalyzed exchange of phosphatidylcholine and cholesteryl ester between plasma lipoproteins

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Abstract A lipid transfer complex (LTC) isolated from human plasma catalyzes equimolar exchange of cholesteryl ester and phosphatidylcholine between low density (LDL) and high density (HDL) plasma lipoproteins. Activation parameters for LTC-catalyzed exchange of neutral and polar lipid are equal and are not influenced by the degree of purity of the catalyst. Activation parameters for exchange of both cholesteryl ester and phosphatidylcholine are influenced by the extent of saturation of phosphatidylcholine fatty acyl groups. The activation parameters also depend on the amount of HDL present in the assay. The flux rates of lipid exchange depend on the concentration of both LDL and HDL. At constant HDL concentration, flux rates become independent of LDL concentration when the ratio of [LDL]:[HDL] exceeds 9:1 (based on cholesteryl ester); at constant LDL concentration, facilitated LDL,HDL lipid exchange is inhibited at high HDL concentration, suggesting preferential HDL,HDL exchange. Analysis of the dependence of initial lipid exchange rate on LDL concentration at two constant HDL concentrations suggests that, in the reaction pathway, LTC mediates a productive collision (ternary complex) between LDL and HDL. A kinetic mechanism consistent with the data is one in which lipid exchange occurs in a ternary complex consisting of LTC, HDL and LDL. At low HDL concentration, this complex is formed by a random sequential route; at high HDL concentration, the mechanism is ordered sequential since the reactants are an LTC-HDL complex and LDL.—**Ihm, J., D. M. Quinn, S. J. Busch, B. Chataing, and J. A. K. Harmony.** Kinetics of plasma protein-catalyzed exchange of phosphatidylcholine and cholesteryl ester between plasma lipoproteins. *J. Lipid Res.* 1982: **23:** 1328–1341.

Supplementary key words cholesteryl ester exchange • phospholipid exchange • plasma lipid transfer protein

Cholesteryl esters, comprising 70% of the total cholesterol of plasma from fasted individuals, reside primarily in the low density lipoproteins (LDL) with much of the remainder in high density lipoproteins (HDL) (1). Cholesterol enters the circulation primarily in unesterified form and leaves the circulation primarily as cholesteryl ester. The cholesteryl ester is produced via the action of the plasma enzyme lecithin:cholesterol acyltransferase (LCAT, EC 2.3.1.43) (2). The cholesterol-containing substrates for LCAT exist in the plasma lipoprotein fractions that float between d 1.063 and d 1.25 g/ml (3, 4). Following esterification, very low density lipoproteins (VLDL) and LDL are the recipients of the cholesteryl ester from the HDL fraction (5–9). The most compelling evidence that human plasma cholesteryl ester follows this migratory pathway is that in subjects deficient in LCAT, not only do the HDL but also the VLDL and LDL lack cholesteryl ester (10). Addition of LCAT to the plasma of LCAT-deficient individuals results in the appearance of cholesteryl ester in all lipoprotein classes. Moreover, in man, but not in animals in which the hepatic enzyme acylCoA:cholesterol acyltransferase (ACAT, EC 2.3.1.26) is more active, the fatty acyl compositions of the cholesteryl ester of VLDL, LDL, and HDL are very similar, the major ester being cholesteryl linoleate (11). In rats, on the other hand, the VLDL that are secreted from the liver contain a cholesteryl ester fraction rich in cholesteryl oleate, while the principal cholesteryl ester in HDL is cholesteryl linoleate (12).

Regulation of the amount and distribution of cholesteryl ester in human plasma is an important issue. Plasma proteins that facilitate the transfer of cholesteryl ester have been identified, isolated, and partially characterized (13–16). Crucial to an understanding of the

Abbreviations: LDL, low density lipoproteins (d 1.019–1.063 g/ml); HDL, high density lipoproteins (d 1.063–1.121 g/ml): VLDL, very low density lipoproteins (d 1.006 g/ml); LTC, lipid transfer complex; LCAT, lecithin:cholesterol acyltransferase; DPPC, L-α-dipalmitoyl phosphatidylcholine; DLLPC, L-α-dilinolenoyl phosphatidylcholine; PC, phosphatidylcholine; CE, cholesteryl ester; DTNB, dithiobis-2-nitrobenzoic acid; PLEP, phospholipid exchange protein; [^{3}H]CE-lipoprotein, lipoprotein labeled with [^{3}H]cholesteryl ester; e.u., entropy units.

role of cholesteryl ester redistribution in whole body cholesterol homeostasis is an understanding of the molecular interaction between the factor(s) catalyzing cholesteryl ester migration and the cholesteryl ester-containing lipoproteins. The purpose of this investigation is to evaluate in a systematic way the kinetics of transfer of cholesteryl ester and phosphatidylcholine in order to develop a mechanistic scheme for catalysis. The transfer[1] protein complex utilized is that which catalyzes *exchange*[1] of cholesteryl ester and phosphatidylcholine between lipoproteins (16, 17). This complex, which consists primarily of protein and a small amount of lipid (17), has been designated the lipid transfer complex (LTC). The lipoproteins employed are LDL and HDL. LTC-facilitated exchange of both phosphatidylcholine and cholesteryl ester between lipoproteins of these classes is sensitive to the fatty acyl composition of the phospholipid constituents, suggesting that the reaction is influenced by the fluidity of the lipoprotein lipid monolayer. In addition, kinetic parameters for exchange of cholesteryl ester and phosphatidylcholine between HDL and LDL are identical. Based on the data, a kinetic model in which lipid exchange occurs by a sequential mechanism involving a ternary HDL-LTC-LDL complex is proposed. The model suggests that directional flow of cholesteryl esters from HDL to lipoproteins of d < 1.063 g/ml can occur through preferential interaction of LTC with HDL relative to LDL.

EXPERIMENTAL PROCEDURES

Materials

Cholesterol and Triglyceride Test Combination Kits were obtained from Boehringer Mannheim Biochemicals. [1,2-^{3}H(N)]Cholesterol (40–60 Ci/mmol), [1-^{14}C]dipalmitoyl phosphatidylcholine (60–100 mCi/mmol), and Aquasol-2 were purchased from New England Nuclear. L-α-Dipalmitoyl phosphatidylcholine (DPPC) was obtained from Avanti Biochemicals. Liquid chromatography supports were purchased as follows: phenyl-Sepharose, thiopropyl-Sepharose, and concanavalin A (ConA)-Sepharose, from Pharmacia Fine Chemicals; CM-52 cellulose and DEAE-52 cellulose, from Whatman. Silica gel IB2 strips and Aquacide were obtained from J.T. Baker Chemical Company and Cal-Biochem, respectively. The phosphatidylcholine exchange protein was purified from beef liver (18). Purified apoA-I was obtained from Dr. Richard L. Jackson.

[1] The terms "exchange" and "transfer" are used interchangeably throughout to mean bidirectional lipid movement. "Net transfer" refers to the unidirectional movement of lipid.

Isolation of plasma lipoproteins and d > 1.21 g/ml plasma infranatant

Lipoproteins were isolated from the freshly collected plasma of normolipemic, fasted human volunteers by sequential ultracentrifugal flotation in KBr containing 4×10^{-5} M EDTA, pH 7.0 (19). A Beckman type 50.2 Ti rotor was used for all centrifugation steps. The purity of each lipoprotein class was assessed by electrophoresis on agarose (1%, pH 8.6) and by immunodiffusion against antibodies raised against LDL, HDL, and apoB, apoE, apoA-I, apoA-II, and serum albumin. LDL were isolated between d 1.019 and d 1.063 g/ml by centrifugation for 18 hr at 48,000 rpm. KBr was added to the d 1.063 g/ml infranatant to increase the density to 1.21 g/ml, and the solution was centrifuged for 24 hr at 48,000 rpm to float lipoproteins of the high density class. The infranatant, excluding the clear zone immediately below the lipoprotein layer, termed the d > 1.21 g/ml infranatant, was employed as the source of the lipid transfer complex for most of the experiments outlined. Lipoproteins and d > 1.21 g/ml infranatant were stored at 4°C in plasma density buffer (10 mM Tris-HCl containing 0.15 M NaCl, 0.02% NaN_3, and 1 mM EDTA, pH 7.4); all lipoproteins were dialyzed immediately prior to use.

Preparation of radiolabeled LDL and VLDL

To prepare double-labeled VLDL and LDL, cholesteryl esters and the phospholipids were labeled sequentially. Freshly isolated plasma (35 ml) was incubated at 37°C for 72 hr with 1 mCi of [1,2-^{3}H(N)]cholesterol impregnated on a Whatman No. 1 filter paper disc (20). Sodium azide (0.02%) was included in the incubation medium. At the conclusion of the incubation, the density of the solution was adjusted with KBr, and LDL were obtained by sequential ultracentrifugation in KBr at solvent densities 1.006, 1.019, and 1.063 g/ml, employing a Beckman 50 Ti rotor operating at 48,000 rpm for 18–24 hr at 15°C. About 65–75% of the radioactivity in the lipoproteins was cholesteryl ester, the remainder being cholesterol. The radiolabeled unesterified cholesterol was removed by exchange with unlabeled cholesterol of lipoproteins of a different density class. LDL were incubated with a 20-fold excess (cholesterol) of human HDL for 6 hr at 37°C. The mixtures were then centrifuged at the appropriate densities to reisolate the [^{3}H]CE-lipoproteins. The VLDL and LDL typically had a radioactivity of 7,000 cpm/μg total cholesterol. In general, less than 4% of the radioactivity occurred as unesterified cholesterol.

[^{3}H]CE-Lipoproteins were labeled with [^{14}C]DPPC by incubation with [^{14}C]DPPC liposomes. To prepare

the liposomes, 10 mg of phospholipid and 10 μCi of [1-^{14}C] dipalmitoyl phosphatidylcholine were mixed in chloroform in a round-bottomed flask; the solvent was evaporated under N_2. The lipid was resuspended by addition of 5 ml of 10 mM Tris-HCl, pH 7.4, containing 0.15 M NaCl and 1 mM EDTA (Tris-buffered saline). The resulting suspension was sonicated at 45–50°C for 15 min (or until clear) with a Sonicator Model W-225R (Heat Systems-Ultrasonics, Inc.) operating at an output of 50–70 W. [^{3}H]CE lipoproteins (3.0 mg of phospholipid), dialyzed overnight against plasma density buffer, were incubated at 44°C for 40 min with 2.5 mg of sonicated [^{14}C]DPPC-liposomes, 2 mg BSA, and 50 μg of bovine liver phospholipid exchange protein. Following incubation, the mixture containing VLDL or LDL was loaded onto a Biogel A15m column that had been packed to 1.6 × 110 cm and equilibrated with plasma density buffer. Elution was performed at room temperature in the same buffer at a flow rate of 25 ml/hr. Fractions containing double-labeled VLDL or LDL, which were well-separated from lipid vesicles, BSA, and PLEP, were pooled and concentrated by ultracentrifugation in KBr. Of the total radioactivity added, 20–25% was recovered in VLDL or LDL. The lipid composition of the lipoproteins was determined before and after radiolabeling, and was unchanged. The percent of the phosphatidylcholine that was DPPC was determined by radioactivity.

Purification of the lipid transfer complex (LTC)

The lipid transfer complex, also referred to as the plasma exchange protein, was isolated from the d > 1.21 g/ml infranatant of human plasma by sequential chromatography on phenyl-Sepharose, CM-cellulose, and concanavalin A-Sepharose essentially as outlined by Ihm et al. (17) except that a thiopropyl-Sepharose step was introduced prior to the ConA-Sepharose step. The fractions containing the phosphatidylcholine and cholesteryl ester exchange activities obtained from CM-cellulose were pooled, and the protein was dialyzed at 4°C against 2 × 4 l of plasma density buffer. The dialyzed protein (about 800 mg) was applied to a thiopropyl-Sepharose column (1.5 × 12 cm) at 4°C equilibrated with the same buffer. The protein that did not bind to the column was collected, dialyzed to 10 mM Tris-HCl, 1 mM $CaCl_2$ (pH 7.4), and applied directly to ConA-Sepharose. The protein in this fraction does not catalyze the exchange/transfer of triglyceride.[2] In addition, for some experiments, the active fraction from ConA-Sepharose was chromatographed on DEAE-cellulose. A DEAE-cellulose column (1.5 × 20 cm) was equilibrated with 10 mM Tris-HCl, pH 6.8, containing 100 mM NaCl, and 3 mg of protein was applied. After the unbound protein was eluted, the bound proteins were eluted with a linear salt gradient of 100 to 300 mM NaCl. All the lipid exchange activity eluted between 0.125–0.175 mM NaCl. The addition of both the thiopropyl-Sepharose and the DEAE-cellulose steps increases the fold-purification to 17,000.

The ConA-purified and the DEAE-purified lipid exchange proteins typically catalyze the exchange of 330 and 420 nmol of cholesteryl ester per hr and 350 and 430 nmol of phosphatidylcholine per hr per mg protein, respectively, between [^{14}C]DPPC/[^{3}H]CE-LDL (100 nmol phospholipid) and HDL (800 nmol phospholipid).

Assay of the lipid transfer complex

The standard assay for exchange of phosphatidylcholine and cholesteryl ester was conducted in plasma density buffer at the specified temperature with [^{14}C]DPPC/[^{3}H]CE-labeled human LDL (usually 50–120 nmol of phospholipid per assay) and human HDL (500–1000 nmol of phospholipid per assay) as lipid donors and acceptors, respectively; the final volume was 1 ml. After the incubation in the absence and presence of LTC, the reactions were terminated by the addition of an equal volume of water (4°C). The assay substrates were reisolated by ultracentrifugation. The density of the incubation mixture was raised to 1.063 g/ml (LDL-HDL assay) or 1.019 g/ml (VLDL-LDL assay) by the addition of 0.32 g and 0.019 g of KBr per ml of solution, respectively. The mixtures were centrifuged for 18 hr at 48,000 rpm in a Beckman 50.3 Ti rotor, and the lipoproteins were isolated and collected. Aquasol (10 ml) was added to 1 ml of each lipoprotein, and amount of radioactivity (^{3}H and ^{14}C) was determined in a Beckman LS-230 liquid scintillation counter equipped with an adjustable discriminator. The nmoles of PC and CE transferred were calculated from the distribution of radioactivity in each lipoprotein, after correcting for recovery of radioactivity. The discriminator was set so that ^{3}H radioactivity counted in the ^{14}C channel was negligible and ^{14}C counts detected in the ^{3}H channel were 15% of ^{14}C counts in the ^{14}C channel. To reduce the counting error, the ratio of ^{3}H to ^{14}C was usually maintained relatively high. Initial rates of lipid exchange vs. LTC concentration were linear for protein values ≤1.5 mg (d > 1.21 g/ml infranatant) or ≤500 μg (LTC purified through the DEAE-cellulose chromatographic step).

Assay of lecithin:cholesterol acyltransferase

The substrate for LCAT was that described previously (17). Briefly, a complex of apoA-I, dilinolenoyl phosphatidylcholine (DLLPC) and [^{3}H]cholesterol was prepared in a molar ratio of 1:100:2. The desired quan-

[2] Ellsworth, J. L., J. A. K. Harmony, and R. L. Jackson. Unpublished data.

tities of DLLPC, [1,2-^{3}H(N)]cholesterol (2 mol %), and organic solvent were dried under N_2. Sufficient Tris-buffered saline was added to give a final DLLPC concentration of 40 mM. The DLLPC-cholesterol mixture (0.25 ml) was mixed with 0.25 ml of apoA-I solution (440 μM) at room temperature, and sufficient sodium cholate (~12.5 mg in 60 μl) was added to clarify the solution. The mixture was then eluted from a column of Biogel P4 (0.9 × 19 cm) to remove the cholate.

The standard assay was conducted using 100-μl aliquots of Tris-buffered saline containing 1 mM dithiothreitol, 500 μg of human serum albumin, 15 μl of the stock substrate solution, and a suitable amount of assay sample. The final volume was 0.1–4.0 ml. The assay mixtures were incubated at 37°C for 6–20 hr. At the end of the incubation, the reactions were terminated by addition of 0.5 ml of methanol. Unesterified and esterified cholesterol were extracted in 4 ml of hexane. The top layer of hexane was withdrawn and evaporated to dryness under N_2. Lipids were redissolved in 0.5 ml of hexane; a 50-μl aliquot was transferred to a scintillation vial to determine total radioactivity; and the remainder was loaded onto a silica gel mini-column. Eluant (2.0 ml of hexane–diethyl ether 6:1 (v/v) was added to the column, and cholesteryl ester was collected directly in a scintillation vial. Mini-columns of silica gel (0.5 × 3.0 cm) were constructed from 14.5-cm Pasteur pipettes; the silica gel was preheated to 100°C for 2 hr.

Calculations

The rate of exchange of PC and CE was calculated assuming that there is exchange only, with no net transfer of mass (17), and that each lipid exists in a kinetically homogeneous pool, all of which is available for exchange. Experimentally determined equilibrium exchange values are 94 and 97% of the theoretically predicted values for CE and PC, respectively. The assumptions are further validated since the relative content of each lipid class (17) and the fatty acyl compositions of the phosphatidylcholine in LDL and HDL, which are nearly identical before incubation, do not change upon incubation with LTC. When LDL with phosphatidylcoline enriched in DPPC are incubated with HDL and LTC, HDL become enriched in DPPC as the proportion of DPPC in LDL decreases; under these conditions, the protein:phosphatidylcholine ratios of the HDL and LDL are not altered.[3] For some experiments, initial rates (<16%) of lipid exchange were determined. In these assays, one substrate (HDL) was maintained at a constant value and one substrate ([^{3}H]CE-LDL) was varied. To compute the initial rate of LTC-facilitated lipid exchange, the initial rate of spontaneous exchange (absence of LTC) was substracted from the initial rate of total exchange (presence of LTC). Reactions in triplicate were monitored for 1 hr; plots of lipid exchange vs. time were linear for at least 2 hr under these conditions. Initial rates of LTC-facilitated lipid exchange were analyzed by the Lineweaver-Burk linear transform. The observed kinetic parameters K_m (the Michaelis constant) and V_{max} (maximal velocity) were calculated by a computerized, weighted linear-least squares fit of the data, where weighting factors directly proportional to V_i^4 were used to prevent artificial biasing of the linear fit due to skewing of the residuals of $1/V_i$ at low V_i. This skewing results from transforming the native data set, V_i vs. S, to $1/V_i$ vs. $1/S$. The rationale for weighting Lineweaver-Burk linear-least squares analysis of enzyme kinetic data has been presented by Cleland (21).

For most studies, the rate constant for lipid exchange that occurs in the absence of LTC—the background exchange (normally less than 5% of the total exchange)—was subtracted from the total rate constant for exchange to analyze the data for LTC-facilitated exchange. The treatment of isotope exchange kinetics summarized by McKay (22) was applied in this work. In the standard assay in which [^{3}H]CE-labeled (or [^{14}C]DPPC/[^{3}H]CE-labeled) LDL are the lipid donors and HDL, the lipid acceptors, a first-order rate expression can be written as follows:

$$-\ln\left[1 - \frac{C_H(t)}{C_H(\infty)}\right] = R\left[\frac{M_H + M_L}{M_H \cdot M_L}\right]t \qquad \text{Eq. } 1)$$

where $C_H(t)$ and $C_H(\infty)$ denote the cpm of CE or PC in HDL at time t(hr) and at equilibrium, respectively; M_L and M_H denote the CE or PC pool sizes (nmol) in LDL and HDL, respectively; R denotes the rate of exchange or flux (nmol/hr) of lipid between LDL and HDL; t is incubation time. The $C_H(\infty)$ was calculated as follows:

$$C_H(\infty) = C_L(0) \cdot \frac{M_H}{M_H + M_L} \qquad \text{Eq. } 2)$$

where $C_L(0)$ represents the cpm of CE or PC in LDL at time 0. The observed first-order rate constant, k_o, in hr^{-1} was determined from the slope of the plot $-\ln[1 - (C_H(t))/(C_H(\infty))]$ vs. t which is equal to $R([M_H + M_L]/[M_H \cdot M_L])$. The rate of exchange, R, was then calculated.

The activation parameters for LTC-facilitated lipid exchange were calculated from the standard relationships. Observed first-order rate constants were converted to units of sec^{-1}. Thus, the energy of activation E_a in kcal/mol was determined from the slope of a plot of $\ln k_o$ vs. $1/T$. Activation parameters were obtained from the Eyring equation:

[3] Miller, D. F. B., and J. A. K. Harmony. Unpublished data.

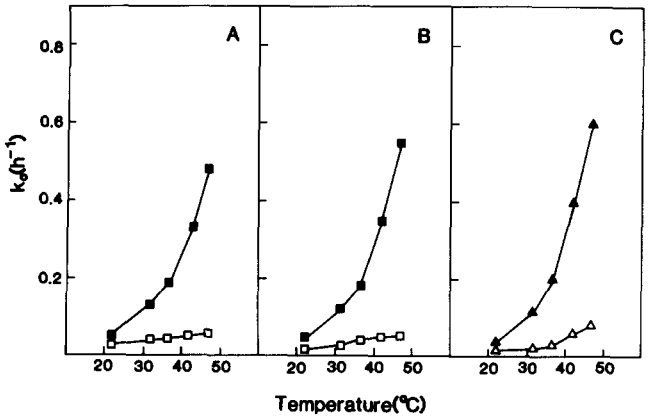


Fig. 1. Temperature dependence of the rate constant, k_o, for exchange of cholesteryl ester and phosphatidylcholine between low density and high density lipoproteins. LDL (120 nmol PC; 180 nmol CE) from a single donor labeled with [^{3}H]CE (A) or [^{14}C]DPPC/[^{3}H] CE (B, C) were incubated with HDL (1980 nmol PC; 590 nmol CE) in the absence (open symbols) or presence (closed symbols) of lipid transfer complex (50 μg protein, purified through the DEAE chromatographic step) for 3 hr in 10 mM Tris-HCl containing 0.15 M NaCl, 0.02% NaN_3, and 1 mM EDTA, pH 7.4. The reaction was quenched at 4°C, and LDL and HDL were separated by ultracentrifugation in KBr, d 1.063 g/ml. The methods for determination of amount of [^{3}H] CE (■ —— □) and [^{14}C]DPPC (▲ —— △) exchanged and the rate constant k_o are described in Experimental Procedures. The amount of DPPC in [^{14}C]DPPC/[^{3}H]CE-LDL was 15% of the total phosphatidylcholine. The experimental error is ±5%, based on duplicate and triplicate determinations.

$$\ln (k_o/T) = (-\Delta H‡/R)(1/T) + \ln k_b/h + \Delta S‡/R \quad \text{Eq. } 3)$$

where k_b and h are the Boltzman and Planck constants, respectively. The free energy of activation, $\Delta G‡$, was calculated at 25°C from the relationship $\Delta G‡ = \Delta H‡ - T\Delta S‡$.

Analytical procedures

Protein concentrations were determined by the method of Lowry et al. (23), modified by the addition of 1% SDS to clarify the samples; bovine serum albumin was the standard. Total triglyceride was determined enzymatically (24) using the Triglyceride Test Combination Kit. Cholesterol and cholesteryl esters were determined by the method of Roeschlau, Bernt, and Gruber (25) using the Cholesterol Test Combination Kit. Total phospholipid was calculated from phosphorus determined by the method of Bartlett (26).

Cholesterol and cholesteryl esters were extracted with ethanol–hexane 40:60 (v/v), and were separated by thin-layer chromatography on silica gel IB2 in a solvent system of hexane–diethyl ether–acetic acid 50:50:1 (v/v). Phospholipids were extracted with 10-fold excess (by volume) chloroform–methanol 2:1 (v/v). Individual phospholipids were separated by thin-layer chromatography on silica gel IB2 in a solvent system of chloroform–methanol–acetic acid–water 25:15:4:2 (v/v). Each phospholipid was identified by comparison with a commercially available standard. All lipids were detected on chromatography plates with iodine vapor. Areas containing lipids were marked and scraped off into scintillation vials, and the radioactivity was determined by liquid scintillation in 10 ml of Aquasol. Phospholipids on silica gel were solubilized with 0.1 ml of chloroform, and the amount of phospholipid in each band was determined as phosphorus. The fatty acids of phosphatidylcholine were quantitated, after liberation by acid hydrolysis, by gas–liquid chromatographic analysis of the methyl esters using a column of 10% EGGS-X (0.002 × 1.8 m). Methyl heptadecanoate acid was the calibration standard. Methyl esters were prepared by incubation of the fatty acids with sodium methoxide (1 ml) for 30 min at 65°C, followed by addition of BF_3 (1 ml, 14%) and incubation for an additional 10 min.

RESULTS

Incubation of LDL and HDL in the presence of the lipid transfer complex results in the exchange of cholesteryl ester and phosphatidylcholine between the two lipoproteins without concomitant net transfer of either neutral or polar lipid (17). The exchange of cholesteryl ester and/or phosphatidylcholine between [^{3}H]CE-LDL or [^{14}C]DPPC/[^{3}H]CE-LDL and HDL was investigated over the temperature range 23–48°C. The [^{14}C]DPPC/[^{3}H]CE-LDL mixture, with 15% of the total phosphatidylcholine as dipalmitoyl phosphatidylcholine (DPPC), was prepared from the [^{3}H]CE-LDL so that a direct comparison could be made of the ability of the two substrates to exchange cholesteryl ester with HDL. As is evident in **Fig. 1,** the rate constants for cholesteryl ester and phosphatidylcholine exchange in the absence of LTC (background, open symbols) are small at all temperatures (for example, 0.017 hr^{-1} and 0.049 hr^{-1} at 23°C and 48°C, respectively, for cholesteryl ester exchange as is shown in Fig. 1B). The rate of lipid exchange is increased markedly by addition of LTC (closed symbols), with the greatest enhancements, 6- to 10-fold above background, achieved at 37–48°C. In Fig. 1, the rate constants for background and total (background plus facilitated) lipid exchange are presented to emphasize the catalytic potency of LTC and to provide information about the magnitude of the spontaneous lipid exchange reaction. Treatment of the data normally involves subtraction of the background rate constant for lipid exchange from that for total lipid exchange to obtain the rate constant for LTC-facilitated lipid exchange.

Arrhenius and Eyring plots were constructed from

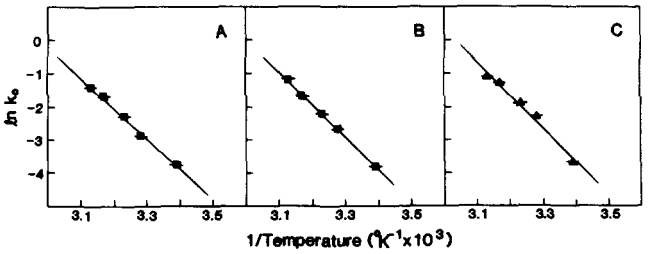


Fig. 2. Arrhenius plots for exchange of CE (■——■) and PC (▲——▲) between [^{3}H]CE-LDL (A) or [^{14}C]DPPC/[^{3}H]CE-LDL (B, C) and HDL facilitated by the lipid transfer complex. The k'_o values for LTC-facilitated lipid exchange are calculated from the data of Fig. 1 by subtracting the rate constant for spontaneous lipid exchange (□ —— △) from that for total lipid exchange (facilitated plus spontaneous, ■ —— ▲) at each temperature. The linear regression analysis correlation coefficients are 0.998–0.999.

the data of Fig. 1, and activation parameters for spontaneous lipid exchange and facilitated lipid exchange were calculated as described in Experimental Procedures. The Arrhenius and Eyring plots constructed from the data were linear and without discontinuities for the entire temperature range as is exemplified by the data illustrated in **Fig. 2,** the Arrhenius plots for LTC-facilitated lipid exchange. The activation parameters for spontaneous and LTC-facilitated exchange are presented in **Table 1.** Three features of the data are noteworthy. First, the energies, enthalpies, and entropies of activation for spontaneous and LTC-facilitated lipid exchange are different while the free energies of activation, $\Delta G‡$, for the two processes are quite similar, about 21.6–22.4 kcal/mol. Second, values of E_a or $\Delta H‡$ for spontaneous exchange of cholesteryl ester are distinct from those for exchange of phosphatidylcholine, while these parameters for LTC-facilitated exchange of the two lipids are, within experimental error, identical. This suggests that the same mechanism accounts for facilitated exchange of phosphatidylcholine and cholesteryl ester, while mechanisms for spontaneous exchange of the lipids are different. Third, activation parameters for LTC-facilitated exchange of cholesteryl ester between [^{3}H]CE-LDL and HDL and between [^{14}C]DPPC/[^{3}H]CE-LDL and HDL are statistically different such that exchange of cholesteryl ester between [^{3}H]CE-LDL and HDL occurs with a more favorable (lower) $\Delta H_F‡$ and a less favorable (more negative) $\Delta S_F‡$ compared to values for exchange between [^{14}C]DPPC/[^{3}H]CE-LDL and HDL. This difference suggests that incorporation of DPPC into LDL influences the exchange of cholesteryl ester. [^{3}H]CE-LDL that were incubated with DPPC vesicles in the absence of the bovine liver phospholipid exchange protein necessary for incorporation of DPPC into LDL behaved identically with control [^{3}H]CE-LDL when incubated with HDL and LTC (data not shown).

The discovery that the activation parameters for exchange of cholesteryl ester and phosphatidylcholine facilitated by LTC are identical reflects the fact that the rate constants are nearly equal at all temperatures, as is evident in Fig. 2. Using the substrates [^{14}C]DPPC/[^{3}H]CE-LDL and HDL, the flux rate of lipid exchange (in nmol/hr) of cholesteryl ester and phosphatidylcholine was determined at each temperature for the spontaneous (**Fig. 3A,** open symbols) and the LTC-facilitated (Fig. 3B) lipid exchange processes. It is evident that the neutral lipid and polar lipid are exchanged with a stoichiometry of 1:1 in both the spontaneous and LTC-facilitated processes at all temperatures between 23° and 48°C. The data thus confirm the previous reports (16, 17) that LTC catalyzes the exchange of cholesteryl ester and phosphatidylcholine with equimolar stoichiometry, and that this stoichiometry is maintained for a variety of reaction conditions and is independent of the state of purification of LTC.

TABLE 1. Activation parameters for exchange of phosphatidylcholine and cholesteryl ester: spontaneous and protein-facilitated exchange

		Activation Parameters							
		Arrhenius Activation Energy		Gibbs Activation Energy		Activation Enthalpy		Activation Entropy	
Assay System	Lipid Exchanged	Ea_B	Ea_F	$\Delta G_B‡$	$\Delta G_F‡$	$\Delta H_B‡$	$\Delta H_F‡$	$\Delta S_B‡$	$\Delta S_F‡$
		kcal mol^{-1}						*cal mol^{-1}K^{-1}*	
[^{3}H]CE-LDL and HDL	CE	5.9	16.6	21.8	21.6	5.3	17.1	−55.5	−15.1
[^{14}C]DPPC/[^{3}H]CE-LDL and HDL	CE	6.8	18.5	22.1	21.6	8.7	18.9	−44.9	−9.2
	PC	17.0	19.7	22.4	21.7	13.9	19.0	−28.4	−9.0

[^{3}H]CE-LDL (180 nmol CE) and HDL (590 nmol CE) or [^{14}C]DPPC/[^{3}H]CE-LDL (180 nmol CE; 120 nmol PC) and HDL (590 nmol CE; 1080 nmol PC) were incubated at 23–48°C for 3 hr either in the absence (for spontaneous exchange denoted by the subscript B) or in the presence of LTC (50 μg protein, purified through the DEAE chromatographic step). The [^{14}C]DPPC/[^{3}H]CE-LDL used for this particular experiment were enriched by 15% with DPPC. Protein-facilitated exhange (denoted by the subscript F) was calculated by subtracting spontaneous exchange rate constant from that for total lipid exchange which occurred in the presence of LTC. Activation parameters were obtained either from Arrhenius plots (E_a), from Eyring plots ($\Delta H‡$, $\Delta S‡$) or from the equation $\Delta G‡ = \Delta H‡ - T\Delta S‡$ where T = 298 K ($\Delta G‡$) as described in Experimental Procedures. The values are precise to ±5% (E_a and $\Delta H‡$) or ±15% ($\Delta S‡$).

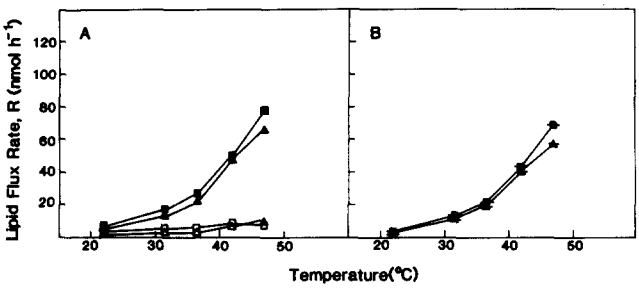


Fig. 3. Dependence on temperature of the flux rate, R, for lipid exchange between [^{14}C]DPPC/[^{3}H]CE-LDL and HDL. The experimental conditions are defined in the legend to Fig. 1. (A) The flux rates for exchange of cholesteryl ester (■ —— □) and phosphatidylcholine (▲ —— △) in the absence (open symbols) and presence (closed symbols) of LTC (50 μg). (B) The flux rates calculated for LTC-facilitated exchange of cholesteryl ester (■——■) and phosphatidylcholine (▲——▲). The methods for determining the rates of lipid exchange are described in Experimental Procedures. The experimental error is ±5%.

To assess the dependence of the activation parameters on the extent of purification of LTC, the temperature dependence of LTC-facilitated exchange of cholesteryl ester between [^{3}H]CE-LDL and HDL was determined in the presence of LTC preparations of low and high specific activity. The activation parameters are presented in **Table 2.** The E_a and the $\Delta H_F‡$ and $\Delta S_F‡$ values obtained are nearly the same for LTC purified only 41-fold from the d > 1.21 g/ml infranatant by chromatography on phenyl-Sepharose or 10,000-fold through four purification steps. Moreover, the activation parameters are not influenced by delipidation of the phenyl-Sepharose-purified LTC with butanol–diisopropyl ether 40:60 (v/v) as described by Cham and Knowles (27). Delipidation removed 34.7 μg of phospholipid and 46.9 μg of cholesterol (total) from 29 mg of LTC protein. Delipidated phenyl-Sepharose-purified LTC (29 mg) retained 19.5 μg of phospholipid and 28.1 μg of cholesterol. The activation parameters reported in Table 2 agree reasonably well with those reported in Table 1 for exchange of CE between [^{3}H]CE-LDL and HDL that were obtained with lipoprotein substrates isolated from a different donor and LTC purified almost 16,000-fold through five chromatographic steps. The relatively small differences in activation parameters in the two situations may be due to differences in the chemical compositions of the substrates.

To examine the importance of lipoprotein concentration in the exchange mechanism, the stoichiometry of phosphatidylcholine and cholesteryl ester exchange was calculated using [^{14}C]DPPC/[^{3}H]CE-LDL, HDL and LTC purified 10,000-fold through the ConA-Sepharose chromatographic step. The data in **Table 3** establish that stoichiometric (equimolar) exchange of cholesteryl ester and phosphatidylcholine is a characteristic of the reaction and is relatively independent of the HDL:LDL ratio which varies from 0.48 to 4.76:1 (CE:CE). The apparent exception is a phosphatidylcholine–cholesteryl ester stoichiometry of 1.64 at the HDL:LDL ratio of 4.76:1. At this HDL:LDL ratio, the error in measuring facilitated exchange was, however, significantly greater than that encountered at the other HDL:LDL ratios, such that the difference in stoichiometry is discounted. Accepting that equimolar exchange of neutral and polar lipid occurs at all HDL:LDL ratios, the dependence of the lipid flux rate on the concentrations of lipoprotein substrates was determined for a single lipid, cholesteryl ester. As is indicated in **Fig. 4,** at a constant amount of HDL (60 nmol of cholesteryl ester), the rate of LTC-facilitated cholesteryl ester exchange increases to an extent proportional to the increase in amount of LDL-cholesteryl ester, and then begins to level off above 125 nmol cholesteryl ester. Saturation, not yet achieved at 250 nmol LDL-choles-

TABLE 2. Activation parameters for protein-mediated exchange of cholesteryl ester: influence of lipid donor and acceptor and of purity of plasma exchange protein

		Activation Parameters			
Assay System	Lipid Exchange Protein	Ea_F	$\Delta G_F‡$	$\Delta H_F‡$	$\Delta S_F‡$
			kcal mol^{-1}		*cal mol^{-1} K^{-1}*
[^{3}H]CE-LDL → HDL	ConA-Sepharose	19.0	22.3	18.4	−13.0
	Phenyl-Sepharose	17.4	21.5	17.8	−12.5
	Phenyl-Sepharose (dl)	18.6	21.7	18.0	−12.4

[^{3}H]CE-LDL (25 nmol CE) and HDL (320 nmol CE) were incubated at 23–48°C for 2 hr in the presence of LTC (75 μg protein purified through the ConA-Sepharose chromatographic step), or 1.9 mg protein purified only through the phenyl-Sepharose chromatographic step, or 1.7 mg protein obtained from phenyl-Sepharose then delipidated (dl) with a mixture of butanol–diisopropyl ether 40:60 (v/v) as described by Cham and Knowles (27). Incubations of the assay substrates in the absence of LTC were performed as a control. The observed rate constants, k'_0, for LTC-facilitated CE exchange were determined, Arrhenius plots and Eyring plots were constructed, and activation parameters were calculated as outlined in Experimental Procedures. The data, precise to ±5% (E_a and $\Delta H‡$) or ±10% ($\Delta S‡$), represent the average of two determinations.

TABLE 3. Stoichiometry of exchange rates for phosphatidylcholine and cholesteryl ester catalyzed by the plasma exchange protein

Assay System	LDL	HDL	HDL:LDL Ratio	Phosphatidylcholine: Cholesteryl Ester
	nmol CE			*mol:mol*
[^{14}C]DPPC/[^{3}H]CE-LDL → HDL	58	28	0.48	1.16
	58	56	0.97	0.96
	58	140	2.41	0.83
	58	276	4.76	1.64
				Average 1.15 ± 0.35

[^{14}C]DPPC/[^{3}H]CE-LDL (58 nmol CE; 41 nmol PC) were incubated at 42°C for 2 hr with various amounts of HDL (mol ratio of PC to CE was 1.54) in the absence or in the presence of LTC (60 μg protein, purified through the ConA-Sepharose chromatographic step). After incubation, LDL were separated from HDL by ultracentrifugation in KBr, d 1.063 g/ml. The exchange rates for PC and CE were calculated as described in Experimental Procedures. The experimental error for triplicate determinations is ±5% except for the HDL:LDL ratio of 4.7:1 where it is ±13%.

teryl ester where the LDL:HDL (CE:CE) ratio is 4:1, occurs at the LDL:HDL ratio of 9:1 (data not shown). In contrast, as is demonstrated in **Fig. 5,** at a constant amount of LDL (180 nmol of cholesteryl ester), the rate of cholesteryl ester exchange increases with increasing HDL-cholesteryl ester only until the amount reaches 100 nmol cholesteryl ester (LDL:HDL, 1.8). For HDL-cholesteryl ester levels greater than 100 nmol, the rate of cholesteryl ester exchange decreases with increasing HDL. A similar kinetic situation has been reported by Barter and Jones (28) using impure LTC (i.e., the d > 1.21 g/ml infranatant) as the source of the exchange activity. The onset of the inhibitory phase in [HDL] seems to depend on the ratio of LTC to HDL rather than on the ratio of lipid donor to acceptor.[4] The effect of increasing both LDL and HDL at constant ratio (1:3 LDL-CE:HDL-CE) was also investigated, and the results are presented in **Fig. 6.** Increasing both substrates at constant ratio increases the rate of exchange of cholesteryl ester. The data for Figs. 4–6 were obtained at 42°C; an identical dependence of flux rate on [LDL], [HDL] or the LDL:HDL ratio exists at 37°C.

To understand further the mechanism of lipid exchange facilitated by LTC and the importance of the LDL:HDL ratio to the kinetics of the exchange reaction, experiments designed to measure cholesteryl ester exchange were performed at different amounts of LDL and HDL, either in the presence or in the absence of LTC. Only the data for LTC-facilitated exchange are reported, and these appear in **Table 4.** Initially, the amount of both [^{3}H]CE-labeled LDL and HDL was increased, with the ratio of LDL to HDL held constant at 1:3 (CE:CE). HDL were then held constant at a value of 180 nmol cholesteryl ester and the amount of LDL was increased from 120 to 240 nmol cholesteryl ester. Finally, activation parameters were determined for the situation in which the ratio of LDL:HDL was 1:9 (CE:CE). Analysis of the data revealed only one direct correlation: the $\Delta H_F‡$ and $\Delta S_F‡$ values increase linearly with increasing amount of HDL regardless of the LDL:HDL ratio, and this relationship is illustrated in **Fig. 7.** In addition, as the amount of HDL increases, the $\Delta G_F‡$ of the reaction decreases proportionately.

Lipid exchange reactions catalyzed by tissue phospholipid exchange proteins have been treated as enzyme-bireactant systems, and the mechanism of such systems has been probed by appropriate velocity studies

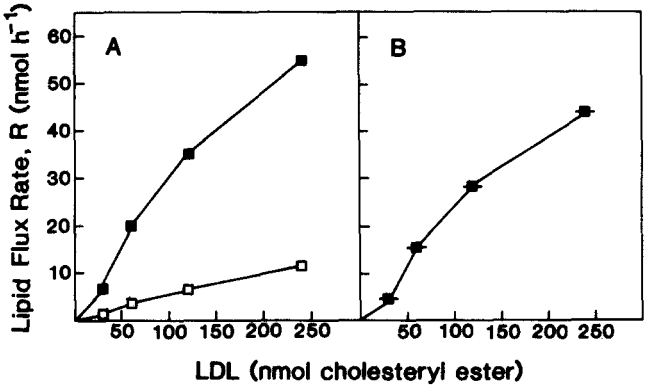


Fig. 4. Dependence of the rate of exchange of cholesteryl ester on the concentration of LDL-cholesteryl ester at constant HDL-cholesteryl ester. For (A), HDL (60 nmol CE) were incubated at 42°C with different amounts of [^{3}H]CE-LDL (30–240 nmol CE) either in the absence (□ —— □) or in the presence (■ —— ■) of LTC (65 μg protein, purified through the ConA-Sepharose chromatographic step). Other reaction conditions are described in the legend to Fig. 1. After 3 hr, LDL and HDL were separated by ultracentrifugation in KBr at d 1.063 g/ml. The nmol of CE transferred were calculated from the distribution of radioactivity in each lipoprotein class; the CE flux rate, R, was calculated as described in Experimental Procedures. (B) The rate of LTC-facilitated CE exchange (■——■) was calculated from the data in (A) by subtracting the background CE flux obtained in the absence (□ —— □) from the total CE flux (■ —— ■). The experimental error for duplicate determinations is ±5%.

[4] Ihm, J., and J. A. K. Harmony. Unpublished data.

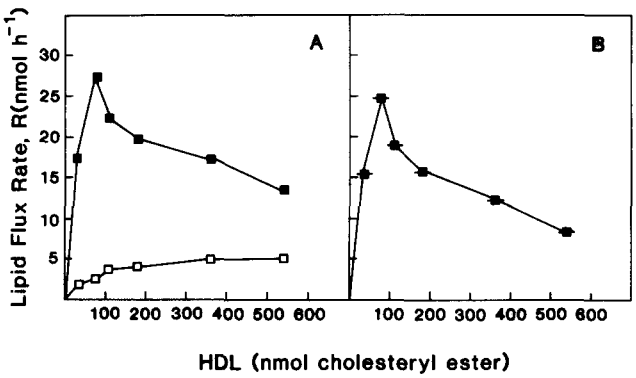


Fig. 5. Dependence of the rate of exchange of cholesteryl ester on the concentration of HDL-cholesteryl ester at constant LDL-cholesteryl ester. For (A), [^{3}H]CE-LDL (60 nmol CE) were incubated at 42°C with different amounts of HDL (30–540 nmol CE) either in the absence (□ —— □) or in the presence (■ —— ■) of LTC (65 μg protein, purified through the ConA-Sepharose chromatographic step). After 3 hr, LDL and HDL were reisolated and the nmol of CE transferred were calculated from the distribution of radioactivity in each lipoprotein class. The rate of flux of CE was calculated as described in Experimental Procedures. (B) The rate of LTC-facilitated CE exchange (-■——■-) was calculated from the data in (A) by subtracting the background flux obtained in the absence of LTC (□ —— □) from the total CE flux (■ —— ■). The experimental error for triplicate determinations is ±5% except at the two highest HDL concentrations where it is ±10%.

(29). Experiments were performed to distinguish a ping-pong type of exchange mechanism in which the exchange protein acts as a shuttle of lipids between donor and acceptor from a sequential mechanism in which the exchange protein mediates a productive interaction between donor and acceptor. The rates of LTC-facilitated

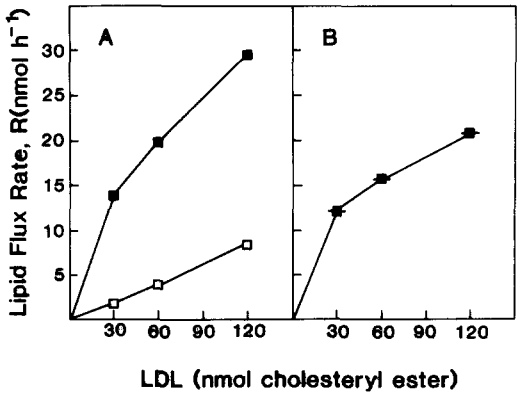


Fig. 6. Dependence of the rate of exchange of cholesteryl ester on the concentration of LDL- and HDL-cholesteryl ester at constant LDL:HDL ratio. For (A), various concentrations of [^{3}H]CE-LDL (30–120 nmol CE) and HDL (90–360 nmol CE) at the fixed ratio LDL-CE to HDL-CE of 1:3 were incubated either in the absence (□ —— □) or in the presence (■ —— ■) of LTC (65 μg protein, purified through the ConA-Sepharose chromatographic step). After 3 hr at 42°C, LDL and HDL were separated by ultracentrifugation and the CE flux rate, R, was calculated as described in Experimental Procedures. (B) The rate of LTC-facilitated CE exchange (-■——■-) was calculated from the data in (A) by subtracting the background CE flux obtained in the absence of LTC (□ —— □) from the total CE flux (■ —— ■). The experimental error is ±5%.

TABLE 4. Protein-facilitated exchange of cholesteryl ester: dependence of activation parameters on the amount of lipid donor and lipid acceptor

LDL	HDL	LDL:HDL Ratio	$\Delta G_F‡$[a]	$\Delta H_F‡$	$\Delta S_F‡$
nmol CE		*mol:mol*	*kcal mol^{-1}*		*cal mol^{-1} K^{-1}*
30	90	1:3	21.1	17.1	−13.5
120	360	1:3	18.4	18.9	+1.7
120	180	2:3	21.4	18.2	−10.8
240	180	4:3	21.3	17.4	−13.2
60	540	1:9	17.2	21.0	+12.8

[a] Calculated at 25°C.

[^{3}H]CE-LDL and HDL were incubated in the absence and presence of LTC (80 μg, purified through the ConA-Sepharose chromatographic step) for 3 hr at temperatures from 23° to 48°C. The activation parameters for LTC-facilitated exchange of cholesteryl ester were calculated by the Eyring method as outlined in Experimental Procedures. The data are averaged from two experiments with the percent errors: $\Delta H‡$, ±5%; $\Delta S‡$, ±12%.

exchange of cholesteryl ester were determined for increasing amounts of LDL at two constant values of HDL, 60 and 180 nmol of cholesteryl ester. The data, analyzed by the Lineweaver-Burk transform as 1/initial rate vs. 1/LDL, are plotted in **Fig. 8.** The double-reciprocal plots are linear and intersect at a common point on the 1/R axis. From the data of Fig. 8, apparent K_m and V_{max} values can be determined. When the amount of HDL is constant at 60 nmol of cholesteryl ester, the

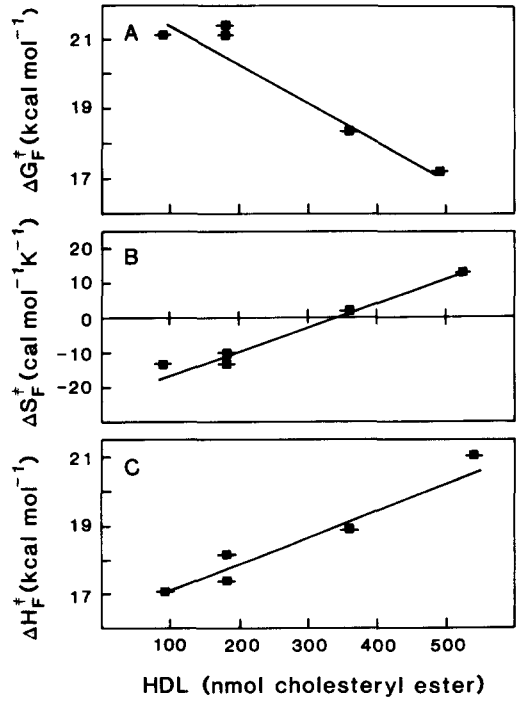


Fig. 7. Dependence of activation parameters for LTC-catalyzed exchange of cholesteryl ester between LDL and HDL on the amount of HDL present in the assay. The data are taken from Table 4. (A) $\Delta G_F‡$; (B) $\Delta S_F‡$; (C) $\Delta H_F‡$.

K_m for LDL-cholesteryl ester is 7 nmol (±3.7%) and the maximum velocity is 158 nmol (±3.2%) of cholesteryl ester exchanged per hr. At constant HDL-cholesteryl ester of 180 nmol, K_m and V_{max} are 12 nmol (±4.8%) LDL-cholesteryl ester and 197 nmol (±4.4%) cholesteryl ester exchanged per hr, respectively. The kinetic parameters are calculated assuming that kinetically active cholesteryl ester in LDL is equal to total amount of cholesteryl ester. Since the appropriate quantity of cholesteryl ester may be that which exists at the lipoprotein surface, a problem inherent in the treatment of heterogeneous catalysis, these values must be considered approximate at best. Moreover, kinetic parameters vary with the preparations of LTC and lipoproteins, although similar experiments performed with three separately purified LTC preparations provided data in which double reciprocal plots converged, within experimental uncertainty, on the 1/rate axis.

The data presented thus far imply an important role for HDL in the kinetic mechanism. To investigate the possible importance of LDL, the influence of increasing the amount of a disaturated phospholipid, dipalmitoyl phosphatidylcholine, in LDL on LTC-facilitated exchange of both phosphatidylcholine and cholesteryl ester was tested. The experiment was prompted by the apparent effect of 15% enrichment in LDL-DPPC on the activation parameters for cholesteryl ester exchange reported in Table 1. The Arrhenius activation energies and the values of $\Delta G_F‡$, $\Delta H_F‡$, and $\Delta S_F‡$ for facilitated exchange of phosphatidylcholine and cholesteryl ester between LDL and HDL are listed in **Table 5.** To minimize errors, the experiments to yield these values were performed in duplicate with LDL isolated from a single donor but enriched to differing extents with DPPC. It is evident from the data that both $\Delta H_F‡$ and $\Delta S_F‡$ increase dramatically with increasing LDL-DPPC, $\Delta H_F‡$ from 17.4 to about 30 kcal/mol and $\Delta S_F‡$ from about 13.8 to 22.5–27.0 e.u. The increase in the enthalpic and entropic activation terms, however, almost balance such that the $\Delta G_F‡$ increase is small with increasing LDL-DPPC: from no enrichment in DPPC to 50% enrichment in DPPC, $\Delta G_F‡$ is between 21.5 and 23.1 kcal/mol.

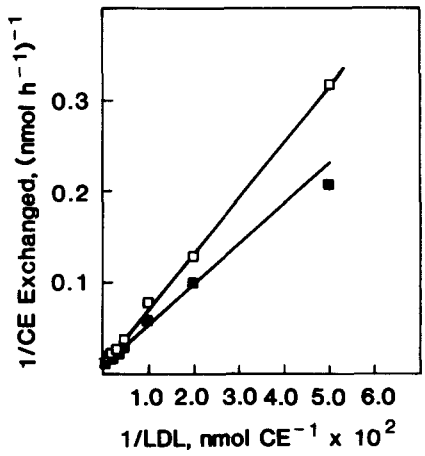


Fig. 8. LTC-mediated exchange of cholesteryl ester between LDL and HDL: dependence of initial exchange rate on the concentration of LDL at constant HDL. HDL, at 60 (■ —— ■) or 180 (□ —— □) nmol cholesteryl ester, were incubated at 37°C for 1 hr with increasing concentrations of LDL in the presence and absence of LTC (100 μg protein purified through the ConA-Sepharose chromatographic step). Initial rate vs. LDL concentration data for LTC-facilitated lipid exchange are analyzed by the Lineweaver-Burk transform as outlined in the Experimental Procedures.

DISCUSSION

The lipid transfer protein complex, LTC, isolated from human plasma catalyzes the *exchange* of phosphatidylcholine and cholesteryl ester between LDL and HDL since, given the assay conditions specified herein, LTC action does not alter the chemical compositions of the substrates (17). With lipoproteins, net transfer of lipid is likely to occur only when the pool size of one or both lipids changes concomitantly with lipid transfer (15, 30, 31). Kinetic analysis of lipid exchange reveals that rate constants for facilitated exchange of zwitterionic phosphatidylcholine and neutral cholesteryl ester are equal at temperatures between 23°C and 48°C. Thus, the activation parameters for LTC-catalyzed exchange of the two lipids are identical within the limits of precision of the assay. This situation exists at all LDL:HDL ratios, and the activation parameters themselves are not influenced by the degree of purity of the catalyst, LTC. Since the values of the sums of the reciprocals of the lipid pool sizes in HDL and LDL [$1/M_H + 1/M_L$ equivalent to $(M_H + M_L)/(M_H \cdot M_L)$ in Equation *1*] for cholesteryl ester and phosphatidylcholine are nearly equal in the assay comprised of HDL and LDL, the flux rates, R, for exchange of the two lipids are also equal at all temperatures. Moreover, the flux rates depend on the concentration of both lipid donor and acceptor. As the amount of LDL increases, the rates of lipid exchange show a phase of first-order dependence on [LDL] and a phase of saturation in which the flux rate is independent of [LDL]. The dependence of the flux rate of lipid exchange on the amount of HDL is complex, being first-order in [HDL] only at low HDL concentrations. At high levels of HDL, the facilitated exchange of phosphatidylcholine and cholesteryl ester between LDL and HDL is inhibited.

The interaction of LTC with lipoproteins is influenced by the lipid composition of the lipoprotein surface. Incorporation of dipalmitoyl phosphatidylcholine into LDL causes profound increases in the activation

TABLE 5. Activation parameters for protein-mediated exchange of phosphatidylcholine and cholesteryl ester between LDL and HDL: influence of LDL-dipalmitoyl phosphatidylcholine

Dipalmitoyl Phosphatidylcholine Enrichment	Lipid Transferred	Activation Parameters			
		Ea_F	$\Delta G_F‡$	$\Delta H_F‡$	$\Delta S_F‡$
%			*kcal mol^{-1}*		*cal mol^{-1} K^{-1}*
0	CE	16.6	21.6	17.1	−15.1
0	CE	17.3	21.4	17.7	−12.4
15	CE	18.5	21.6	18.9	−9.2
15	PC	19.7	21.7	19.0	−9.0
20	CE	21.9	22.4	19.4	−10.2
20	PC	19.4	22.1	18.8	−11.2
30	CE	19.0	22.3	18.4	−13.0
30	PC	23.2	22.0	22.6	+1.9
35	CE	23.4	22.4	22.6	+0.7
35	PC	26.7	22.2	25.9	+12.6
40	CE	22.6	23.0	22.6	−1.5
40	PC	27.9	23.1	27.7	+15.5
50	CE	30.1	22.7	29.4	+22.5
50	PC	31.2	22.5	30.5	+27.0

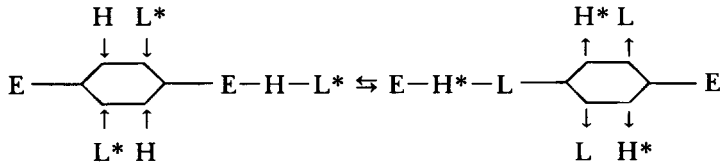
LDL (180 nmol CE) labeled with [^{14}C]DPPC and/or [^{3}H]CE were incubated with HDL (590 nmol CE) for 3 hr at 23°–48°C in the absence or presence of LTC (50 μg, purified through the DEAE chromatographic step). [^{14}C]DPPC/[^{3}H]CE-LDL enriched with various amounts of DPPC were prepared by incubating [^{3}H]CE-LDL with sonicated [^{14}C]DPPC vesicles and bovine liver phospholipid exchange protein as described in Experimental Procedures. The rate constants for LTC-facilitated PE and CE exchange were calculated and Eyring plots were constructed as outlined in Experimental Procedures. Activation parameters, $\Delta H_F‡$ and $\Delta S_F‡$, were determined from the Eyring plots, and $\Delta G_F‡$ was calculated at 298 K. The activation parameters were calculated for LTC-facilitated lipid exchange only. The experimental error for duplicates is <10%.

parameters for exchange of both neutral and polar lipid between LDL and HDL: $\Delta H_F‡$ and $\Delta S_F‡$ increase by 12.6 kcal/mol and approximagely 38 e.u., respectively, as the percent of disaturated phospholipid increases from ~0% to 50%. The increase in $\Delta H_F‡$ may reflect an increase in the energy barrier to movement of lipids into and out of the lipoprotein since incorporation of dipalmitoyl phosphatidylcholine into LDL decreases the fluidity of the hydrophobic core of these particles (16). Helmkamp (32) concluded that bovine brain phospholipid exchange protein (PLEP) is also sensitive to the fluidity of the lipid-containing substrates. A decrease of the fluidity of the phospholipid in sonicated liposomes increased the $\Delta H‡$ for catalyzed liposome-microsome phospholipid exchange by 6.2 kcal/mol (calculated at 25°C from the data of reference 32). Although the kinetic mechanisms for LTC- and PLEP-catalyzed lipid exchange may be different, as suggested below, the molecular movements of the transferred phospholipids may be subject to the same physicochemical perturbations. In the case of transfer of phosphatidylcholine and cholesteryl ester facilitated by LTC, the kinetic consequence of increasing the degree of saturation of the polar lipid is reflected directly in the transfer of cholesteryl ester. This suggests a mechanism whereby the P/S ratio of the diet influences the concentration and distribution of plasma cholesterol (33).

Why are the rate constants for facilitated exchange of phosphatidylcholine and cholesteryl ester between HDL and LDL equal? The lipids that are eligible for LTC-facilitated transfer may exist in a phospholipid:cholesteryl ester complex at the lipoprotein surface. Cholesteryl ester, though conventionally designated as a lipoprotein core lipid, is miscible with phospholipid as shown in both monolayer lipid (34) and bulk lipid (35) systems. On the other hand, equal rate constants for exchange of phosphatidylcholine and cholesteryl ester may arise as a consequence of the structural composition of LTC. The LTC preparation employed in this investigation is comprised of two major proteins of apparent molecular weights of 58,000 and 63,000 (17), allowing the possibility that one protein in the complex transports cholesteryl ester and the other, phosphatidylcholine. The rate constants may also be dictated by the lipid composition of LTC (17). These points await resolution. All of the explanations are con-

sistent with the fact that alteration of the phospholipid composition of an LTC substrate alters the kinetics of transfer of the phospholipid as well as the neutral lipid.

Based on the data presented herein, what is a reasonable mechanism for LTC-facilitated exchange of phosphatidylcholine and cholesteryl ester between LDL and HDL? The Lineweaver-Burk plots of Fig. 8 intersect on the y-axis, which suggests that the exchange of lipids between LDL and HDL occurs by a sequential mechanism involving a ternary HDL-LDL-LTC complex. Were exchange to occur by a ping-pong mechanism involving binary HDL-LTC and LDL-LTC complexes, the Lineweaver-Burk plots would be parallel. In addition, the activation parameters of Table 4 and Fig. 7 show the following trends: *a*) there is a sizable decrease in $\Delta G_F‡$ of 4 kcal/mol when [HDL] is increased by a factor of 6; and *b*) there are partially compensating changes in $\Delta H_F‡$ and $\Delta S_F‡$ over the same [HDL] increase. The value of $\Delta H_F‡$ increases by ~4 kcal/mol, but this increase is outweighed by about 26 e.u. increase, such that the change in $T\Delta S_F‡$ makes a contribution of −7.7 kcal/mol. These trends in activation parameters suggest a change in reaction mechanism as [HDL] increases. A proposed mechanistic scheme consistent with these observations is shown below:



Eq. *4*)

$$H \;\; \downuparrows \;\; K_H$$

$$(E-H) \underset{K_L}{\overset{L^*}{\rightleftharpoons}} (E-H)-L^* \rightleftarrows (E-H^*)-L \underset{L}{\overset{K_L}{\rightleftharpoons}} (E-H^*) + L$$

The asterisk indicates the radioactive lipid, E is LTC, H is HDL, L is LDL, K_H is the dissociation constant of the LTC-HDL complex, and K_L is the dissociation constant of LDL from the LTC-HDL-LDL complex. This scheme can also apply to a situation in which net transfer of lipids occurs.

According to this scheme, when [HDL] is low most of the LTC is in its free form (unbound by lipoproteins) and the effective reactant state is free LTC plus free HDL plus free LDL. The complex in which exchange takes place is a ternary complex of LTC, HDL, and LDL, and so (of necessity) is the activated complex or transition state for exchange. The exchange reaction then is formally third-order. However, consider the possibility that as [HDL] increases LTC becomes increasingly committed to formation of a binary LTC-HDL complex, and eventually essentially all the LTC is in this complex. We now have a new reactant state, the LTC · HDL complex plus free LDL. However, the transition state is still the same since it involves a ternary LTC · HDL · LDL complex. The reaction is now formally second-order since the effective reactant state has changed. The large increase in $T\Delta S_F‡$ of 7.7 kcal/mol attending the increase in [HDL] is consistent with a change in mechanism from third-order to second-order. Bruice and Benkovic (36) reported increases in $T\Delta S‡$ of 4–6 kcal/mol for each decrease in kinetic order for nucleophilic displacement reactions on phenyl acetates, and the Sackur and Tetrode equation (37) predicts a decrease of $T\Delta S‡$ of 2–3 kcal/mol for translation of two molecules to form a complex. Steinberg and Scheraga (37) calculated a decrease of $T\Delta S‡$ of 2.7 to 5.5 kcal/mol (−9 to −18 e.u.) when two proteins interact to form a complex wherein interaction is restricted to a fraction of their surfaces. As they discussed, increases in solvent entropy will probably compensate (at least partially) for this entropy cost of complex formation. It should be pointed out that the observed increase in $\Delta S_F‡$ with increasing [HDL] of 26 e.u., which is ascribed by Equation *4* to a change from third-order to second-order of the reaction, also likely contains contributions from changes in solvent entropy. Since for the third-order LTC reaction the increase in solvent entropy in proceeding from the reactant to the transition state should be greater than for the second-order reaction, the increase in $\Delta S_F‡$ from contributions other than solvations should be greater than 26 e.u. Both reaction manifolds in the scheme of Equation *4* involve sequential reactions. The top manifold is written as random sequential because this is the most general case of a sequential mechanism and the available data do not allow postulation of a more specific ordered sequential mechanism. At high [HDL] the mechanism is ordered sequential since

LTC is saturated with HDL. The kinetic mechanism for LTC-facilitated lipid exchange is therefore distinct from that for exchange of phosphatidylcholine between lipid donor and acceptor catalyzed by the phospholipid exchange proteins from bovine brain and liver (38). These proteins act as shuttles of lipid between donor and acceptor via a ping-pong mechanism.

It must be pointed out that the temperature dependence experiments from which the activation parameters were calculated gauge the reaction thermodynamics of the LTC-catalyzed LDL, HDL exchange only. The plots of Fig. 5 show that the exchange rate increases with increasing [HDL] to a maximum, with inhibition of exchange occurring at higher value of [HDL]. This may reflect an increasing commitment of LTC to HDL, HDL exchange at high [HDL] through formation of a ternary LTC·HDL·HDL complex. Barter, Ha, and Calvert (7) reported that crude LTC, namely that present in lipoprotein-free plasma, catalyzes the exchange of cholesteryl ester between HDL_2 and HDL_3. Purified LTC also catalyzes the exchange of cholesteryl ester between HDL_2-Sepharose and HDL_2 or HDL_3 and between HDL_3-Sepharose and HDL_2 or HDL_3.[4]

The importance of HDL in the lipid exchange reaction, as indicated by these kinetic studies, provides a rationale for the flow of cholesteryl ester from the HDL density class to lipoproteins of $d < 1.063$ g/ml, the physiologically relevant process (5–7). The molar ratio of LTC to HDL and the affinity of HDL for LTC will determine whether the process is random sequential or ordered sequential. A number of other lines of evidence support the kinetic evidence for an LTC·HDL complex. Pattnaik and Zilversmit (39) reported that the cholesteryl ester exchange activity isolated from human plasma forms an isolable complex with HDL. This complex does not form when the species are incubated at pH 4.5, or at pH 7.5 in the presence of calcium ion (1 M). These conditions, which preclude the physical interaction of LTC with HDL, also drastically reduce the rate of LTC-facilitated exchange of both cholesteryl ester and phosphatidylcholine between HDL and LDL (17).

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