Interaction of Lecithin:Cholesterol Acyltransferase and Cholesteryl Ester Transfer Protein in the Transport of Cholesteryl Ester into Sphingomyelin Liposomes†

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ABSTRACT: When isolated lecithin:cholesterol acyltransferase was incubated with cholesterol–lecithin liposomes in the presence of apolipoprotein A-1, cholesteryl ester accumulated until a maximal ester/lecithin weight ratio of 0.03 was reached. This was independent of the amount of enzyme present or the proportion of cholesterol relative to lecithin. The inhibition of transferase associated with accumulation of cholesteryl ester was relieved by addition of additional lecithin–cholesterol liposomes but not by addition of sphingomyelin liposomes containing the same proportion of substrate unesterified cholesterol. These results indicate that it is the accumulation of cholesteryl ester product which directly inhibits transferase activity. When isolated cholesteryl ester transfer protein from human plasma was included in the reaction mixture, cholesteryl ester was transported to sphingomyelin–cholesterol liposomes, with associated release of transferase from product inhibition. Cholesteryl ester incorporated directly into the liposomes or synthesized from free cholesterol via the transferase reaction was equally transferred to sphingomyelin acceptor liposomes, indicating that the cholesteryl ester in these particles formed a single miscible pool for transfer.

Lecithin:cholesterol acyltransferase (LCAT) (EC 2.3.1.43) catalyzes the formation of cholesteryl esters from lipoprotein lecithin and free cholesterol; reaction of the isolated enzyme with synthetic lecithin–cholesterol liposomes is dependent upon the presence of a specific plasma coprotein (apo A-1) (Fielding et al., 1972), a major protein of plasma high density lipoprotein. The lysolecithin product of the reaction is transferred to albumin while cholesteryl ester accumulates within the substrate liposome (Aron et al., 1978). Addition of cholesteryl ester to the substrate liposomes inhibited LCAT activity, and it was proposed that product inhibition might regulate transferase activity (Fielding et al., 1972). However, in native lipoproteins the cholesteryl ester generated by the LCAT reaction was shown to be transported from the substrate lipoproteins to nonsubstrate acceptors such as the low and very low density lipoprotein complexes (Rehnborg & Nichols, 1964; Nichols & Smith, 1965). This transfer is mediated via a specific cholesteryl ester transfer protein recently isolated from human plasma (Chajek & Fielding, 1978). The functional linkage between inhibition of LCAT by cholesteryl ester and the relief of such inhibition by transfer of cholesteryl ester via the transfer protein has not been hitherto demonstrated. In the present research the relationship between the activities of LCAT, apo A-1, and transfer protein (apo D) has been investigated and the properties of acceptor liposomes have been partially defined.

Materials and Methods

Materials. [1,2-^{3}H]Cholesterol (45–60 Ci/mmol) and [4-^{14}C]cholesterol (40–50 mCi/mmol) were purchased from New England Nuclear, Boston, MA, and unlabeled cholesterol was from Pfanstiel, Waukegan, IL. Cholesterol was repurified before use by thin-layer chromatography on silica gel layers on glass plates (silica gel G, E. Merck, Darmstadt, Germany) developed in cyclohexane–ethyl acetate (6:4 v/v) and extracted into benzene. [^{3}H]Cholesteryl oleate was synthesized with oleyl chloride and labeled cholesterol (Deykin & Goodman, 1962) and repurified by chromatography on silica gel layers developed in benzene–cyclohexane (4:1 v/v) (Fielding et al., 1979). L-α-Dioleylphosphatidylcholine, unlabeled cholesteryl oleate, and recrystallized human serum albumin were from Sigma Chemical Co., St. Louis, MO. DL-Dioleylphosphatidylethanolamine and sphingomyelin (beef brain) were from Serdary Laboratories, London, Ontario. Phospholipids were repurified via thin-layer chromatography on silica gel using chloroform–methanol–NH_4OH–water (70:30:3:2 v/v) and the areas corresponding to pure synthetic standards were eluted with methanol. Diethyl *p*-nitrophenyl phosphate was from K & K Laboratories, Plainview, NY, 2% cross-linked agarose (Bio-Gel A-50M) was from Bio-Rad, Richmond, CA, and activated Sepharose (Sepharose 4B–CNBr complex) and concanavalin A–Sepharose covalent complex were from Pharmacia, Uppsala, Sweden.

Preparation of Soluble and Immobilized Liposomes. Dioleyllecithin or sphingomyelin, mixed with cholesterol (generally in a weight ratio of 8:1), was evaporated from a methanol solution under vacuum and dispersed by vortex mixing in 2–5 mL of glass-distilled water ($>10^5$ Ω cm^{-2}) at a concentration of 0.8 mg/mL phospholipid. The sample was transferred to the microcell (JA-3399) of a French Pressure Cell (American Instrument Co., Silver Springs, MD). The pressure was raised to 20 000 psi/cycle for 3 cycles of 30 s. In some experiments cholesteryl oleate (up to a 0.03 weight ratio to phospholipid) was included in the lipid mixture and liposomes were prepared in the same way. The particles in either case formed a single peak included in the 2% agarose columns of mean diameter (350 Å) slightly greater than that of low density lipoprotein (220 Å) in columns standardized against very low, low, and high density lipoproteins (Sata et al., 1972). For the purpose of binding liposomes to activated Sepharose, particles were prepared from a lipid mixture containing 5% w/w dioleylphosphatidylethanolamine in addition to cholesterol and lecithin in the proportions described; this addition is without effect on the substrate properties of the

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0006-2960/80/0419-3673$01.00/0

LCAT reaction (Aron et al., 1978). A 3.2-mg amount of liposome lecithin was mixed with 0.5 g dry weight equivalent of activated Sepharose which had been washed with 1 mM HCl and then suspended in 0.1 M $NaHCO_3$. The mixture of lipids and Sepharose was incubated at 4 °C for 20 h, filtered on sintered glass, and then resuspended in 0.1 M aqueous ethanolamine (pH 7.4). The complexed lipid (40–50% of that added) was finally recovered and washed by filtration. The bound liposomes had a lipid composition not significantly different from that of the original particles. Liposomes for reaction with LCAT were preincubated for 1 h at 37 °C with 12.5 μg of apo A-1 (Shore & Shore, 1968) per 100 μg of phospholipid in the presence (per mL) of 50 mg of human serum albumin (pH 7.4) (Fielding, 1974) and 10 mM EGTA. Sphingomyelin–cholesterol liposomes for immobilization to Sepharose also contained 5% w/w dioleylphosphatidylethanolamine. These were prepared by the same procedure as that for the lecithin–cholesterol particles.

Preparation of LCAT. LCAT was isolated from human plasma essentially as previously described (Aron et al., 1978). In summary, after fractionation with solid ammonium sulfate (40–66% saturation), this fraction was dissolved in D_2O–14.5% w/v CsCl and mM EDTA and centrifuged to isolate the lipoprotein fraction of density 1.21–1.23 g/cm^3. After a second centrifugation under similar conditions, the enzyme was further purified by column chromatography on Sephadex G-100 and on hydroxylapatite and finally traces of apo D were removed by affinity chromatography on immobilized IgG from the plasma of rabbits immunized against isolated apo D (Aron et al., 1978). The eluate showed a single protein band in several electrophoresis systems and retained both transferase activity with lecithin–cholesterol liposomes and phospholipase activity with pure lecithin liposomes; the product was unreactive with antibodies raised against human apo A-1 and apo D under conditions when a 0.5% contaminant would have been detected. The isolated LCAT (purified about 20000-fold from plasma) was recovered in 8–10% yield.

Preparation of Cholesteryl Ester Transfer Protein. Transfer protein was isolated from human plasma high density lipoprotein ($1.063 > d > 1.21$ g/cm^3) by affinity chromatography on immobilized anti-apo D antibody; the column was equilibrated with 0.01 M Tris-HCl, 0.15 M NaCl, and 1 mM Na_2EDTA (pH 7.4) at 4 °C and a flow rate of 4 mL/h. The absorbed protein was eluted with 3 M NaCNS in distilled water (Chajek & Fielding, 1978). 2-Mercaptoethanol was added (to 20 mM) and the protein solution further fractionated on concanavalin A–Sepharose (1.2 × 20 cm column) previously equilibrated with 1 mM $CaCl_2$–1 mM $MgCl_2$–1 mM $MnCl_2$ in distilled water. The column was washed with 0.15 M NaCl, and the bound protein was then eluted with 0.2 M α-methyl mannoside in distilled water. The eluate (containing 40–50 μg of pure cholesteryl ester transfer protein from an original 10 mg of HDL protein) contained a single protein species by electrophoresis and had a specific activity of 950–1000 nmol of cholesteryl ester transferred from high to low density lipoprotein per mg per h at pH 7.4 and 37 °C, comparable to previous values (Chajek & Fielding, 1978).

Isolation of Apo A-1. Apo A-1 was isolated from human high density lipoprotein ($1.063 < d < 1.21$ g/cm^3), delipidated with ethanol–diethyl ether (2:1 v/v at −20 °C), and fractionated first by chromatography on Sephadex G-150 equilibrated with 0.01 M Tris-HCl and 8 M urea, pH 8.1, then by DEAE-cellulose chromatography using a gradient (0.01–0.1 M) of NaCl in the same buffer. The chemical composition of the product was not significantly different from that previously reported (Shore & Shore, 1968). Additionally, the purified protein showed no reaction with antibodies against human serum apo D.

Other Procedures. Protein was measured with the Folin phenol reagent (Lowry et al., 1951), phospholipid as lipid phosphorus (Bartlett, 1959), and cholesterol and cholesteryl oleate with cholesterol oxidase and cholesterol esterase (Heider & Boyett, 1978). LCAT activity is expressed in terms of the rate of production of labeled cholesteryl ester from liposomes containing [^{3}H]cholesterol (Fielding, 1974). The incubation mixture was extracted with chloroform and methanol (Bligh & Dyer, 1959), and portions of the chloroform phase were taken for automated thin-layer chromatography (AIS Specialties, Libertyville, IL); thin-layer plates of silica gel were developed in hexane–diethyl ether–acetic acid (83:16:1 v/v), and the cholesteryl ester areas (R_f 0.9–0.95) were analyzed for radioactivity in scintillation medium containing 0.5% PPO and 0.03% POPOP in toluene; quenching (which was minimal) was monitored with [^{3}H]toluene. Recovery of chemical and radiochemical cholesteryl ester from this system was essentially complete (>98%). Cholesteryl ester transfer was determined as the rate of transport of cholesteryl ester from soluble cholesterol–lecithin liposomes to immobilized liposomes containing the same proportion of cholesterol. Under these conditions in the absence of added LCAT there was no net movement of cholesterol to or from the lecithin–cholesterol liposomes. In the presence of LCAT, the incubation was terminated by addition of 10^{-4} M diethyl *p*-nitrophenyl phosphate (Rose & Juliano, 1976) and the bound liposomes were recovered by centrifugation at 4 °C for 5 min at 2000*g* or by filtration as described above and washed 5 times with distilled water; under these conditions no loss of phospholipid from the immobilized particles was detected in control experiments without added lecithin liposomes. The lipid composition of the Sepharose-bound particles and of portions of the soluble phase from the incubation assay was determined after extraction with chloroform and methanol and thin-layer chromatography, as described above. When apo A-1 and LCAT were added to the immobilized liposomes and were rapidly separated from the incubation medium by filtration, essentially the whole of the transferase was rapidly associated with the lecithin–cholesterol liposomes used in this study; slightly lower binding was found with pure lecithin liposomes (Figure 1). Of the apo A-1 added to the medium, a maximum of ~80% of that added (8 μg/150 μg of lecithin) was rapidly bound to the liposomes and retained during the filtration and washing procedures (Figure 1).

Results

Inhibition of LCAT Activity by Product Cholesteryl Ester. When LCAT was incubated with lecithin–cholesterol liposomes in the presence of apo A-1, the rate of enzyme activity decreased with time almost to zero, at a point at which the cholesteryl ester content of the particles was ~3.0% w/w with respect to lecithin. When increasing concentrations of enzyme were incubated with liposomes containing the same free cholesterol content, the same maximal level of esterification was reached in each case (Figure 2), even though this was reached at a rate dependent upon the enzyme concentration in the incubation medium. Similar results were obtained when the substrate concentration was modified in relation to a fixed level of added transferase; the same end point was reached in terms of percent cholesterol esterifieid (Figure 3).

This result might have been obtained from irreversible inactivation of the enzyme during incubation. However, as shown in Figure 4, when fresh liposomes were added to the

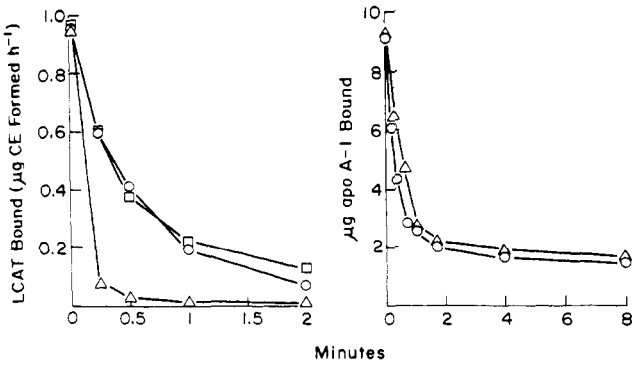

FIGURE 1: Binding of LCAT and apo A-1 to immobilized lecithin or lecithin–cholesterol liposomes. Left panel: LCAT (0.2 μg of protein) was added to lecithin liposomes without apo A-1 (□), lecithin liposomes with apo A-1 (10 μg of protein) (○), or cholesterol–lecithin liposomes (molar ratio 0.1) with apo A-1 (10 μg of protein) (△) in a total volume of 0.4 mL of assay medium containing 0.2 μmol of phospholipid in each case in 0.15 M NaCl, pH 7.4. Incubation was at 37 °C, and the amount of bound transferase at each time point was determined as the difference between original activity and soluble activity recovered after separation of the Sepharose–Liposomes. In the absence of immobilized liposomes, recovery of LCAT activity through the procedure was 100.1 ± 1.3%. Recovery of soluble liposomes under the same conditions was 98 ± 2% (assayed as lipid phosphorus). Right panel Binding of apo A-1 to lecithin (△) and lecithin–cholesterol (○) liposomes. Assay conditions were the same as those described above except for the absence of LCAT. Binding was assayed in terms of protein recovered after filtration relative to original protein; recovery of apo A-1 through the procedure in the absence of liposomes was 98 ± 3%.

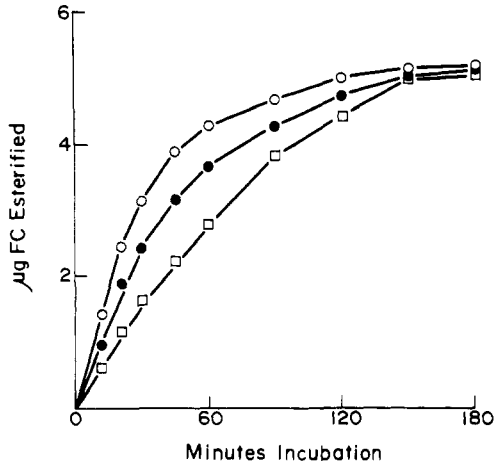

FIGURE 2: Cholesteryl ester synthesis as a function of LCAT concentration. LCAT in the proportions indicated below was added to assay medium containing dioleyllecithin (0.4 μmol), cholesterol (0.06 μmol), apo A-1 (40 μg), and human serum albumin (50 mg) in a reaction volume of 1.12 mL of 0.15 M NaCl, pH 7.4. 0.6 mL of purified LCAT (1.2 μg/mL) or smaller amounts diluted with NH_4HCO_3 buffer (Aron et al., 1978) were added, and 50-μL samples were taken at zero time and at intervals thereafter during incubation at 37 °C. The reaction was stopped with chloroform and methanol, and labeled cholesteryl ester was determined as described under Material and Methods. (○) 0.6 mL of enzyme; (●) 0.4 mL of enzyme; (□) 0.2 mL of enzyme. FC = free cholesterol.

incubation medium, the original esterification rate was established. This activation was not mediated by transfer of free cholesterol from the added to the original liposomes [i.e., the activation of a reduced level of enzyme by modulation of the substrate-free cholesterol/lecithin ratio (Fielding et al., 1972)] since addition of liposomes of cholesterol with sphingomyelin (not a substrate for LCAT) (Fielding, 1974) containing the same cholesterol content did not activate the inhibited transferase activity. Additionally, when liposomes of different

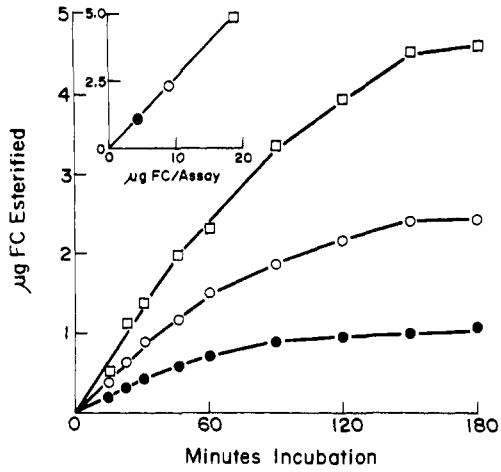

FIGURE 3: Cholesteryl ester synthesis as a function of medium phospholipid concentration. The reaction conditions were the same as those in the legend to Figure 1, except that the volume of enzyme added was 0.2 mL and substrate phospholipid concentrations were 0.4 (□), 0.2 (○), and 0.1 (●) mL/reaction volume. The phospholipid/cholesterol ratio was 6.5:1.

Table I: Effect of Substrate Liposome Lecithin/Cholesterol Molar Ratio on the End Point of LCAT-Mediated Cholesteryl Ester Synthesis

substrate composition (phospholipid/ cholesterol molar ratio)	cholesteryl ester (μg)[a]	% total cholesterol esterified	wt % (relative to phospholipid)
22.3	6.74 ± 0.24	26.8 ± 0.7	2.9 ± 0.1
11.1	6.87 ± 0.24	37.5 ± 1.4	2.6 ± 0.2
6.7	6.93 ± 0.25	60.8 ± 2.2	2.5 ± 0.1

[a] Weight calculated from unesterified cholesterol by using a molecular weight ratio for ester/free cholesterol of 1.72. Each assay contained 268 μg of dioleyllecithin with the indicated weight of unesterified cholesterol and apo A-1, human serum albumin, and EGTA in the proportions shown in the legend to Figure 4. The values obtained after 3 and 4 h of incubation at 37 °C under these conditions differed by <5%, indicating the end point of the reaction. Values are means ± SD of triplicate determinations.

Table II: Effect of Added Cholesteryl Oleate on the End Point of LCAT-Derived Cholesteryl Ester Synthesis[a]

cholesteryl oleate added (μg)	cholesteryl oleate formed (μg)	total cholesteryl ester (μg)
0	8.1 ± 0.2	8.1
0.45	7.5 ± 0.4	8.0
0.90	7.1 ± 0.2	8.0
1.80	6.2 ± 0.2	8.0

[a] Values are means ± SD of triplicate determinations.

cholesterol content (in terms of free cholesterol for a fixed lecithin substrate concentration) were compared in terms of the end point of their reaction with LCAT, a similar limiting value was obtained in each case, even though this was attained by the esterification of different amounts of total free cholesterol (Table I). When cholesteryl ester was incorporated into liposomes before their exposure to LCAT, the amount of cholesteryl ester that could be synthesized by transferase activity prior to inhibition decreased in the same amount, while the total amount of cholesteryl ester (from addition and synthesis) at the point when inhibition was reached remained the same (Table II). These experiments, taken together, indicate that it is the accumulation of product cholesteryl ester which inhibits the further activity of LCAT in liposomes and that this end point is reached when the particles contain ~3%

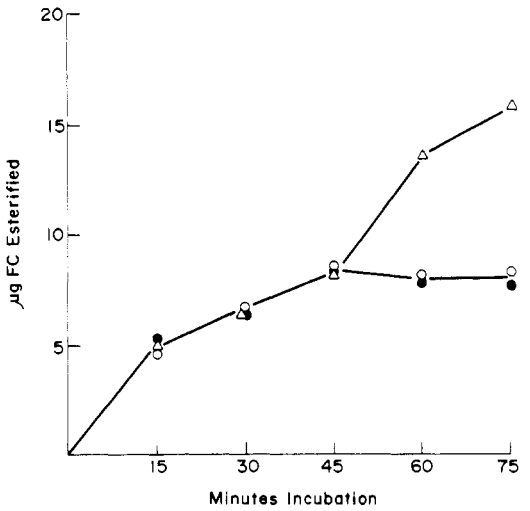

FIGURE 4: Effect of substrate and nonsubstrate liposomes on the inhibition of LCAT by cholesteryl ester. Liposomes containing 400 μg of dioleyllecithin and 50 μg of cholesterol were incubated (37 °C, 1 h) with apo A-1 and albumin in the proportions shown in the legend to Figure 1 in a reaction volume of 1.6 mL. 0.6 mL of purified LCAT (1.0 μg) was added to 1.4 mL of substrate and incubated for 45 min at 37 °C. Samples (0.2 mL) were taken at 15, 30, and 45 min; at this point fresh medium was added to each incubation, containing either liposome-free medium (○) 400 μg of sphingomyelin liposomes containing 50 μg of cholesterol (●) or 400 μg of lecithin and 50 μg of cholesterol (△), to maintain the phospholipid/cholesterol ratio of the medium. Samples were taken at intervals thereafter for determination of medium cholesteryl ester content.

cholesteryl ester by weight of lecithin. The elution pattern of these particles on 2% agarose was not significantly changed from that of the original lecithin–cholesterol liposomes. Finally, accumulation of cholesteryl ester in the liposomes and inhibition of LCAT activity were not associated with release of LCAT from the immobilized liposomes into the medium; less than 1% of added transferase was recovered in the soluble fraction after centrifugation after 3 h of incubation under the conditions shown in Figure 2.

Release of LCAT Inhibition by Cholesteryl Ester Transfer Protein. Liposomes containing lecithin, cholesterol, and apo A-1 were incubated with LCAT until inhibition was reached; then isolated transfer protein and sphingomyelin–cholesterol–apo A-1 liposomes on Sepharose were added. There was a release from inhibition as demonstrated by the initiation of further esterification of free cholesterol (Figure 5). Neither sphingomyelin vesicles alone nor transfer protein alone catalyzed any further synthesis of cholesteryl ester by LCAT with lecithin–cholesterol liposomes. The activity of the transfer protein was associated with the net transport of cholesteryl ester into the acceptor (sphingomyelin) liposomes (Figure 5, insert); indeed, the cholesteryl ester transferred was usually in slight excess over that synthesized by LCAT, resulting in a slight net decrease in the cholesteryl ester content of the substrate (lecithin) liposomes. The end point of transfer in these experiments was reached when the sphingomyelin–cholesterol liposomes contained ~1.5% cholesteryl ester. Analysis of the phospholipid composition of the soluble and bound liposomes showed the absence of any exchange of lecithin and sphingomyelin betwen the vesicles. The transfer of preformed and newly formed cholesteryl ester was compared by use of lecithin–cholesterol liposomes with LCAT such that at the end point of inhibition the particles contained approximately equal amounts of ^{14}C-labeled cholesteryl ester, added to the lipid dispersion in the French press, and ^{3}H-labeled cholesteryl ester, synthesized from [^{3}H]cholesterol via the

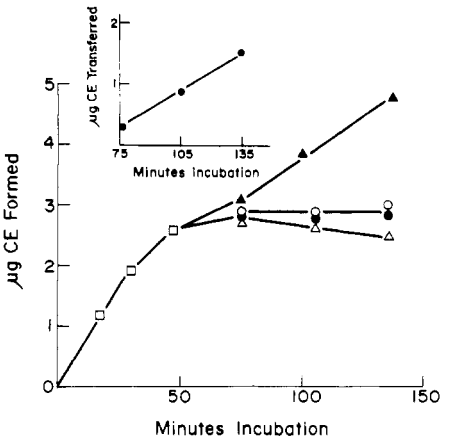

FIGURE 5: Effect of cholesteryl ester transfer protein on cholesteryl ester mediated inhibition of LCAT activity. 400 μg of dioleyllecithin and 50 μg of cholesterol were preincubated at 37 °C for 1 h with apo A-1 and human serum albumin in the proportions listed in the legend to Figure 1. 0.6 mL of LCAT solution was added, and the reaction mixture was incubated for a further 50 min. At this point Sepharose 4B complex covalently linked to either sphingomyelin–cholesterol liposomes (at the same cholesterol/phospholipid ratio as that for lecithin) or ethanolamine (Porath et al., 1967) was added, with or without transfer protein (5.6 μg). 0.3-mL samples were taken at 0, 15, 30, and 45 min after the second addition into ice-cooled tubes containing 50 μL of diethyl *p*-nitrophenyl phosphate (10^{-3} M). The gel was separated from the medium by centrifugation and washed with distilled water, and the level of cholesteryl ester bound to the gel and present in samples of the incubation medium was determined. (□) Lecithin substrate liposomes only; (▲) transfer protein + sphingomyelin vesicles; (○) transfer protein alone; (●) sphingomyelin vesicles alone; (△) soluble fraction from addition of transfer protein + sphingomyelin liposomes. Insert: (●) cholesteryl ester recovered with Sepharose–sphingomyelin covalent complex. CE = cholesteryl ester.

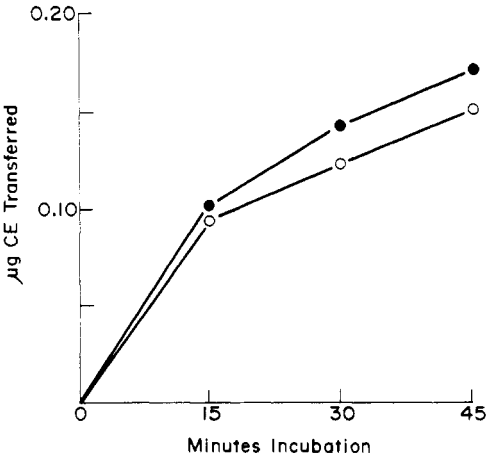

FIGURE 6: Transfer of synthetic and LCAT-derived cholesteryl ester to sphingomyelin-cholesterol liposomes immobilized on Sepharose 4B. 400 μg of dioleyllecithin with 50 μg of [^{14}C]cholesterol (sp act. 6.7 × 10^{4} cpm μg^{-1}) and [^{3}H]cholesteryl oleate (1.3 μg; sp act. 1.64 × 10^{5} cpm μg^{-1}) were incubated with apo A-1 and albumin as described in the legand to Figure 1 in a volume of 2.2 mL. 0.2 mL of LCAT solution was added (0.5 μg). After 45 min (at which time the vesicles contained 1.1 μg of [^{14}C]cholesteryl oleate in addition to the 1.3 μg of synthetic labeled ester), 40 μg of sphingomyelin as sphingomyelin-cholesterol liposomes complexed with Sepharose was added [with the same [^{14}C]cholesterol specific activity as the lecithin (substrate) liposomes] together with 1 μg of transfer protein and 0.1 volume of diethyl *p*-nitrophenyl phosphate to inhibit further LCAT activity. Samples of sphingomyelin-Sepharose liposomes were taken for centrifugation at 0, 15, and 30 min thereafter, and their content of [^{14}C]cholesteryl ester (○) and [^{3}H]cholesteryl ester (●) was determined. The proportion of synthetic to LCAT-derived cholesteryl ester transferred was 1.16 ± 0.03, not significantly different from the ratio (1.15) in the original lecithin-cholesterol liposomes.

LCAT reaction. As shown in Figure 6, the transfer rates for each isotope were essentially identical, indicating that the cholesteryl ester of the liposomes formed a single pool, in terms of reaction with the transfer protein.

Discussion

The present investigation illustrates a number of previously undescribed features of the cholesteryl ester synthesis and transfer reactions. Here it is shown that cholesteryl ester synthesized by isolated LCAT inhibits transferase activity under conditions where the physical properties of the substrate appear to be little changed in terms of binding of apo A-1 or LCAT or retention of phospholipids. This effect is mediated not by depletion of substrate free cholesterol and a change in lecithin/cholesterol substrate ratio nor by inactivation of the enzyme but by the accumulation of cholesteryl ester product itself in the liposomes. Indeed, exogenous and enzymatically synthesized cholesteryl ester had equivalent effects in these experiments. This end point of the transferase reaction is quite similar to the maximal saturation of cholesteryl ester in egg lecithin under these conditions (Janiak et al., 1974, 1979). It is relevant that similar proportions of cholesteryl ester and lecithin are also found in the biological membranes of a tissue (liver) actively involved in the clearance of plasma cholesteryl ester (Zambrano et al., 1975). While native lipoproteins contain much higher levels of cholesteryl ester than this (Skipski, 1972), it has been proposed that this forms an interior "core" of pure oil phase (Sata et al., 1972). However, on physical grounds it seems very likely that the lecithin and cholesterol surface film of these particles would contain cholesteryl ester in equilibrium with the core components such as to contain a saturating concentration of cholesteryl ester, which would account for ~5% of the total cholesteryl ester in high density lipoprotein and ~1% of total cholesteryl ester in low density lipoprotein (the latter calculation was based on the presence of a surface sphingomyelin film). This surface component may be analogous to the cholesteryl ester of the liposomes.

It has been previously shown that cholesteryl ester transfer protein catalyzes net transport from high to low and very low density lipoproteins in whole plasma (Rehnborg & Nichols, 1964; Nichols & Smith, 1965; Chajek & Fielding, 1978). The present study shows that cholesteryl ester synthesis and transfer, at least between lipid vesicles, have no coprotein requirement beyond what is involved in the LCAT reaction (apo A-1). In this research sphingomyelin vesicles were used because this phospholipid is not a substrate for LCAT, because it does not exchange with lecithin under the conditions of these experiments, because when mixed with lecithin liposomes containing the same cholesterol content there is no net transport of sterol, and because it forms a major component of the surface film of the natural acceptor lipoprotein (low density lipoprotein) (Skipski, 1972). Higher proportions of cholesteryl ester are present in the native lipoproteins than can be incorporated into the liposomes. This may be either because the interior of the liposomes is aqueous rather than hydrophobic or because apolipoprotein, although not involved in transfer, plays a role in cholesteryl ester binding; these possibilities are under investigation. The present research strongly indicates that in the liposome system at least, the whole of the cholesteryl ester forms a single miscible pool. It also indicates that cholesteryl ester containing liposomes may form a suitable substrate for studying the biochemistry of the transfer reaction.

The transfer protein used in this study and previously (Chajek & Fielding, 1978) catalyzes net transport of cholesteryl ester (as shown in the present studies where transfer is into vesicles initially free of cholesteryl ester) and little or no exchange of cholesteryl ester between lipoproteins, has a molecular weight of 35 000, and is located primarily in the plasma high density lipoprotein fraction. A cholesteryl ester exchange protein, recently described (Pattnaik et al., 1978), catalyzes exchange apparently without net transport, has a molecular weight of ~80 000, and is located in the $d > 1.25$ g/cm^3 density fraction. The cholesteryl ester transfer and exchange proteins on these grounds appear to represent distinct biochemical entities. The biochemical properties of the transfer reaction studied here appear to be those of the net transport process of whole plasma (Rehnborg & Nichols, 1964; Nichols & Smith, 1965).

Acknowledgments

Stephen Jones provided excellent technical assistance.

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