Interconversion between apolipoprotein A-I-containing lipoproteins of pre-beta and alpha electrophoretic mobilities

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Abstract Apolipoprotein (apo) A-I-containing lipoproteins can be separated into two subfractions, pre-beta HDL and alpha HDL (high density lipoproteins), based on differences in their electrophoretic mobility. In this report we present results indicating that these two subfractions are metabolically linked. When plasma was incubated for 2 h at 37°C, apoA-I mass with pre-beta electrophoretic mobility disappeared. This shift in apoA-I mass to alpha electrophoretic mobility was blocked by the addition of either 1.4 mM DTNB or 10 mM menthol to the plasma prior to incubation, suggesting that lecithin:cholesterol acyltransferase (LCAT) activity was involved. There was no change in the electrophoretic mobility of either pre-beta HDL or alpha HDL when they were incubated with cholesterol-loaded fibroblasts. However, after exposure to the fibroblasts, the cholesterol content of the pre-beta HDL did increase approximately sixfold, suggesting that pre-beta HDL can associate with appreciable amounts of cellular cholesterol. Pre-beta HDL-like particles appear to be generated by the incubation of alpha HDL with cholesteryl ester transfer protein (CETP) and either very low density lipoproteins (VLDL) or low density lipoproteins (LDL). This generation of pre-beta HDL-like particles was documented both by immunoelectrophoresis and by molecular sieve chromatography. Based on these findings, we propose a cyclical model in which *1)* apoA-I mass moves from pre-beta HDL to alpha HDL in connection with the action of LCAT and the generation of cholesteryl esters within the HDL, and *2)* apoA-I moves from alpha HDL to pre-beta HDL in connection with the action of CETP and the movement of cholesteryl esters out of the HDL. Additionally, we propose that the relative plasma concentrations of pre-beta HDL and alpha HDL reflect the movement of cholesteryl esters through the HDL. Conditions that result in the accumulation of HDL cholesteryl esters will be associated with low concentrations of pre-beta HDL, whereas conditions that result in the depletion of HDL cholesteryl esters will be associated with elevated concentrations of pre-beta HDL. This postulate is consistent with published findings in patients with hypertriglyceridemia and LCAT deficiency.—**Kunitake, S. T., C. M. Mendel, and L. K. Hennessy.** Interconversion between apolipoprotein A-I-containing lipoproteins of pre-beta and alpha electrophoretic mobilities. *J. Lipid Res.* 1992. **33:** 1807–1816.

Supplementary key words pre-beta HDL • alpha HDL • lecithin: cholesterol acyltransferase • cholesteryl ester transfer protein

High density lipoproteins (HDL) are a collection of discrete species that vary in composition and structure. For the most part, the functions of these species are ill-defined; however, it is possible that each may perform a unique task in the body. One of the chief processes in which HDL appear to participate is reverse cholesterol transport (1, 2), the multi-step process in which cholesterol is transported from the periphery to the liver. This process may involve several HDL species.

One way that HDL species can be differentiated is on the basis of their electrophoretic mobility. We have previously shown that immunoisolated apoA-I-containing lipoproteins (Lp(A-I)) can be divided into two major subfractions, those with alpha electrophoretic mobility, "alpha HDL", and those with pre-beta mobility, "pre-beta HDL" (3).[2]

We first isolated and characterized pre-beta HDL by preparative electrophoresis after isolation of Lp(A-I) from plasma (3). Although the majority of apoA-I-containing lipoproteins in plasma are alpha HDL, pre-beta HDL are also normal components of human plasma. Observations made earlier on serum (4) and centrifugally isolated HDL

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; LCAT, lecithin:cholesterol acyltransferase; CETP, cholesteryl ester transfer protein; Lp(A-I), apoA-I-containing lipoprotein; TBS, Tris-buffered saline; PBS, phosphate-buffered saline.

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[2]The precise definition of HDL is those lipoproteins ultracentrifugally isolated between the density interval 1.063 to 1.21 g/ml. Lipoproteins isolated by selected affinity immunosorption all contain apoA-I. As apoA-I is the dominant protein in HDL, we used HDL as shorthand for the immunoisolated particles. We then named the alpha HDL and pre-beta HDL subfractions based on their general electrophoretic mobilities. These terms are not intended to imply any structural feature or lipid content to the two subfractions.

(5, 6) are consistent with the presence of pre-beta HDL as we have defined them. They are also present in the plasma of mice (7), monkeys (8), and dogs (9). Particles similar to pre-beta HDL have been identified in the plasma of patients with lecithin:cholesterol acyl transferase (LCAT) deficiency (10, 11), in the plasma of patients undergoing chronic hemodialysis (12), and in the medium of cultured HepG2 cells (13). The concentration of pre-beta HDL in plasma can range from virtually nonexistent in patients who have undergone small bowel resection (K. LaSala, S. T. Kunitake, and J. P. Kane, unpublished results) to more than half of the apoA-I mass in the plasma of hypertriglyceridemic subjects (14–16), although the average concentration of pre-beta HDL is about 5% of the apoA-I in the plasma of normolipidemic individuals (14, 16). Furthermore, pre-beta HDL have been postulated to serve a key role in the process of reverse cholesterol transport, possibly acting as the initial acceptors of cellular unesterified cholesterol (17–21).

We have shown that pre-beta HDL are small, protein-rich lipoproteins that are composed predominantly of apoA-I, without detectable apoA-II, and structurally distinct from alpha HDL (3, 22). Subsequently, three subfractions of apoA-I-containing lipoproteins whose electrophoretic mobilities differ from the electrophoretic mobility of alpha HDL, when resolved by two-dimensional gel electrophoresis, have been identified (18, 19). During preparative isolation of pre-beta HDL by starch block electrophoresis, only the peak fractions are recovered; particles with mobilities differing from the peak pre-beta HDL are excluded, possibly accounting for differences in the description of pre-beta HDL species. Using this method, the vast majority of pre-beta-migrating apoA-I, isolated from the plasma of fasting normolipidemic subjects, appears to reside in particles of approximately 60 kDa (S. T. Kunitake, C. M. Mendel, and L. K. Hennessy, unpublished results).

Pre-beta HDL and alpha HDL appear to be metabolically linked. We have reported on the movement of pre-beta HDL to alpha HDL when plasma is incubated (23). In the present study we investigated the interconversion of apoA-I between pre-beta HDL and alpha HDL. Specifically, we monitored the movement of apoA-I mass between pre-beta HDL and alpha HDL apparently linked to the movement of cholesteryl esters into and out of the total pool of plasma HDL (HDL compartment).

METHODS

Isolation of lipoproteins

Blood was drawn from fasting normolipidemic individuals (males and females) and immediately mixed with the following preservatives to give the indicated concentrations: ethylene-diaminetetraacetic acid (EDTA) (0.08% W/V), sodium azide (0.1% W/V), benzamidine (500 μg/ml), phenylmethanesulfonyl fluoride (10 μg/ml), ϵ-aminocaproic acid (3.0 mg/ml), and gentamicin sulfate (10 μg/ml). Plasma was separated by low speed centrifugation at 1000 *g* for 45 min at 4°C and stored at 4°C for up to 1 week. Alpha-2-macroglobulin (10 μg/ml) in addition to the above-listed preservatives was added at all steps in lipoprotein isolation, to all isolated lipoproteins, and was present in all of the following experiments to prevent lipoprotein degradation.

Apolipoprotein A-I-containing lipoproteins (Lp(A-I)) were isolated by selected affinity immunosorption (24–26), as previously described. Briefly, plasma was applied to an immunoaffinity column made with antibodies directed against human apoA-I and pre-selected for their ability to dissociate from apoA-I under the chosen elution conditions. Unbound proteins were washed from the column with 150 mM NaCl, 5 mM Tris, pH 7.4 "wash buffer" (TBS). Subsequently, all of the bound Lp(A-I) were eluted with 150 mM NaCl, 0.2 M acetic acid, pH 3.0. The eluate was neutralized with 2 M Tris, concentrated under nitrogen (20 psi) with an Amicon ultrafiltration cell fitted with a YM 10 membrane (Danvers, MA), and exchanged into TBS. The eluate was then passed through protein A-Sepharose and anti-human albumin-Sepharose columns for removal of contaminants.

Pre-beta HDL and alpha HDL were prepared by starch block electrophoresis of Lp(A-I), as previously described (3). Briefly, potato starch was hydrated with 50 mM barbital, pH 8.6, and poured into a Plexiglass form. After removal of excess buffer, the starch formed a rigid block. The Lp(A-I) sample was loaded onto the cathodic end of the block and an electric potential was applied across the block. After the lipoproteins had migrated sufficiently, the block was fractionated into 1-cm segments and the apoA-I content of each fraction was determined by immunonephelometry (27). The pre-beta HDL and alpha HDL were recovered from the appropriate fractions. The purity of the pre-beta HDL and alpha HDL was evaluated by immunoelectrophoresis (28) in 1% agarose with monospecific goat antiserum directed against apoA-I (Tago, Burlingame, CA) and by SDS polyacrylamide gel electrophoresis (26).

Lipoproteins were also prepared by sequential ultracentrifugation (29) in a Beckman preparative ultracentrifuge with a 40.3 rotor and running conditions of 35,000 rpm and 4°C for 24 h. Very low density lipoproteins (VLDL) were prepared directly by the centrifugation of plasma. Low density lipoproteins (LDL) were isolated between the density interval of 1.006 and 1.063 g/ml using KBr to adjust the background density. A d 1.063 g/ml top fraction was isolated by adjusting plasma directly to 1.063 g/ml. Centrifugations were performed twice at each density. The lipoprotein fractions were dialyzed against TBS and stored at 4° for up to 2 weeks.

Incubation of plasma and plasma components

Plasma was incubated for 2 h in a shaking water bath at 37°C. The presence of pre-beta HDL and alpha HDL in plasma before and after incubation was determined by immunoelectrophoresis (28) with antiserum directed against apoA-I. In some experiments, isolated alpha HDL and pre-beta HDL were radioiodinated by the iodine monochloride method, as modified for lipoproteins by Bilheimer, Eisenberg, and Levy (30), mixed with plasma, and either stored at 4°C or incubated for 2 h at 37°C, and then analyzed by agarose electrophoresis.

The LCAT inhibitors, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB, 1.4 mM) and menthol (10 mM), were added separately to plasma samples prior to incubation to determine the effect of LCAT activity on the conversion of pre-beta HDL to alpha HDL.

Lp(A-I) (100 μg protein) alone, Lp(A-I) plus d $<$ 1.063 g/ml lipoproteins (100 μg protein), and plasma reconstituted by recombining bound and unbound fractions (plasma concentrations) from the anti-apoA-I selected-affinity immunosorption column were incubated for 2 h at 37°C and analyzed by immunoelectrophoresis.

To determine the effect on HDL of transfer of cholesteryl esters from HDL to acceptor lipoproteins, alpha HDL (100 μg protein) were mixed with cholesteryl ester transfer protein (CETP, 8 μg) and either VLDL or LDL (100 μg protein) and incubated for 5 h at 37°C. The mobilities of the apoA-I-containing lipoproteins in the incubation mixture were then determined by immunoelectrophoresis with monospecific antisera directed against either apoA-I (Tago, Burlingame, CA) or apoA-II (International Immunology, Murietta, CA). The incubation mixture was also examined for the generation of particles with the size of pre-beta HDL by molecular sieve chromatography with a Superose 12 column (1 × 30 cm, Pharmacia, Uppsala, Sweden) eluted with TBS buffer at a flow rate of 0.4 ml/min.

Incubation of pre-beta HDL and alpha HDL with cholesterol-loaded cells

Human newborn foreskin fibroblasts in their 10th to 15th passages were grown under 8% CO_2 in 6-cm diameter tissue culture dishes (Costar, Cambridge, MA) in Dulbecco's modified Eagle medium H21 (DME), supplemented with 2 mM glutamine and containing 50 μg/ml gentamicin and 10% fetal calf serum. When the cells reached near confluence, the medium was changed to DME containing 10% lipoprotein-deficient serum (d $>$ 1.25 g/ml, 60 mg protein/ml, apoA-I free), cholesterol (50 μg/ml, added from a solution of 15 mg/ml in ethanol), and Sandoz compound 58-035 (an inhibitor of acyl CoA:cholesterol acyltransferase, 5 μg/ml). Twenty four h later the cells were washed three times with Dulbecco's phosphate-buffered saline (PBS) containing 0.5% human serum albumin (Cutter Laboratories, Emeryville, CA) and three times with PBS alone as described previously (31).

Two ml of DME containing either pre-beta HDL or alpha HDL (250 μg protein/ml) and the above-listed preservatives (excluding ETDA and azide) were incubated with the cells for 2 h at 37°C. After incubation the medium was recovered and the lipoproteins were reisolated by selected-affinity immunosorption directed against apoA-I. The electrophoretic mobilities of the lipoproteins were determined by immunoelectrophoresis; their protein contents before and after incubation were determined by the method of Lowry et al. (32); and their cholesterol contents were measured by an enzymatic assay (33).

RESULTS

The incubation of whole plasma for 2 h at 37°C resulted in the disappearance of pre-beta HDL (**Fig. 1**). There was no net loss of apoA-I mass from the incubated plasma (159 ± 3 mg/dl compared to 158 ± 3 mg/dl after incubation) and the radioactivity of ^{125}I-labeled pre-beta HDL appeared to shift to alpha mobility when pre-beta HDL was incubated with plasma (**Fig. 2**), indicating that the apoA-I with pre-beta electrophoretic mobility apparently converted to alpha mobility. ApoA-I can exchange between pre-beta HDL and alpha HDL; however, if pre-beta HDL were still present after incubation, a small peak of radioactivity should have been detected in the pre-beta zone. The incubation of either pre-beta HDL or alpha HDL alone did not alter their electrophoretic mobility, as determined by immunoelectrophoresis and the migration of radiolabeled HDL (3), suggesting that there was no artifactual change in mobility due to the incubation. A decrease in the free cholesterol content of the incubated plasma was observed (data not shown).

When either of two LCAT inhibitors, DTNB or menthol, was added to the plasma prior to incubation, the pre-beta HDL remained visible (**Fig. 3**). As DTNB and men-

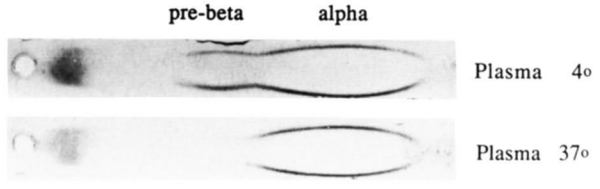

Fig. 1. Incubation of plasma. Plasma from a fasting normolipidemic subject was either stored at 4°C (top) or incubated for 2 h at 37°C (bottom). The plasma was then analyzed by immunoelectrophoresis with antiserum directed against apoA-I. Zones of alpha and pre-beta electrophoretic mobility are indicated. These findings are representative of the results obtained from the incubation of plasmas from more than ten subjects.

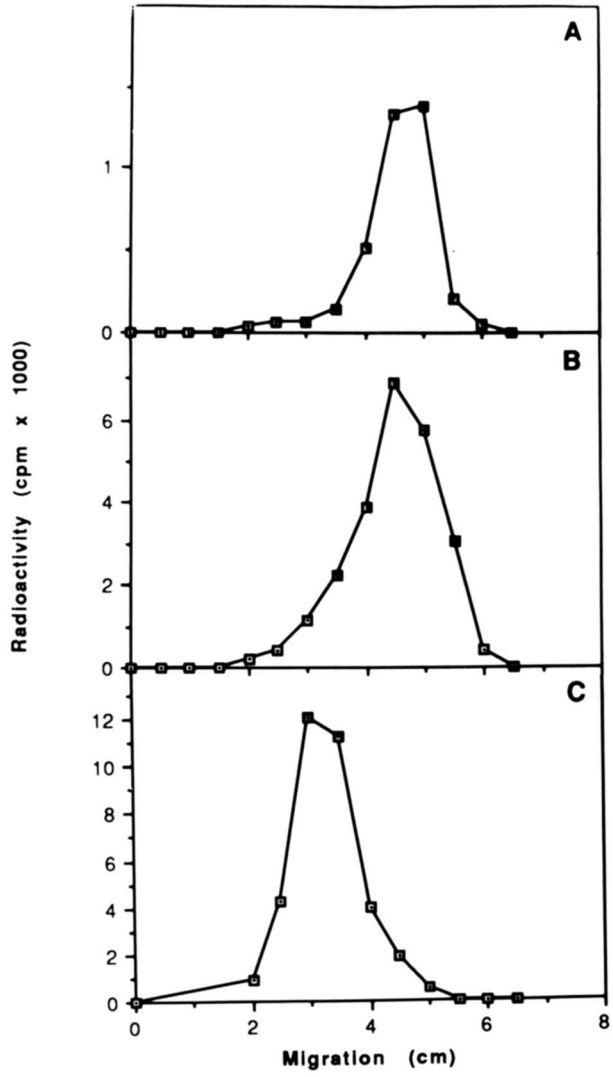

Fig. 2. Incubation of pre-beta HDL with plasma. Isolated pre-beta HDL were radioiodinated then mixed with plasma and incubated for 2 h at 37°C. The samples were subjected to agarose electrophoresis, the agarose was cut into segments, and the radioactivity in each segment was measured. (A) Alpha HDL; (B) pre-beta HDL mixed with plasma and incubated at 37°C; (C) pre-beta HDL mixed with plasma and stored at 4°C.

thol apparently inhibit LCAT by different mechanisms, their ability to block the disappearance of pre-beta HDL suggests an association between the conversion of pre-beta HDL into alpha HDL and cholesterol esterification.

Incubation of neither Lp(A-I) alone nor Lp(A-I) in the presence of LDL and VLDL resulted in the disappearance of pre-beta HDL within the 2-h incubation period (**Fig. 4**). It was only when the bound (Lp(A-I)) and unbound (the rest of the plasma components) plasma fractions from the anti-apoA-I selected-affinity immunosorption column were recombined and incubated that the pre-beta HDL disappeared (data not shown). This finding suggests that an additional plasma factor, such as

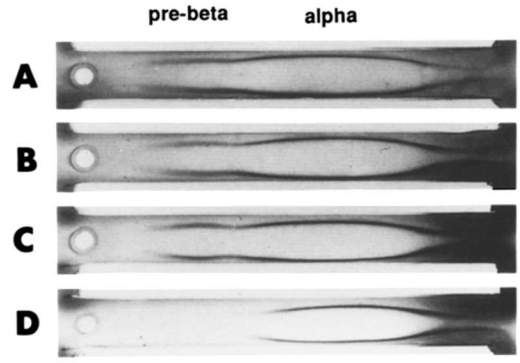

Fig. 3. Incubation of plasma in the presence of LCAT inhibitors. Plasma was mixed with either 1.4 mM DTNB or 10 mM menthol and incubated for 2 h at 37°C. After incubation, the mixtures were analyzed by immunoelectrophoresis with antiserum directed against apoA-I. (A) Plasma stored at 4°C; (B) plasma containing 1.4 mM DTNB incubated at 37°C; (C) plasma containing 10 mM menthol incubated at 37°C; (D) plasma incubated for 2 h at 37°C. These findings are representative of the results obtained in three separate experiments.

the factor proposed by Rye and Barter (34), may help to facilitate the disappearance of pre-beta HDL.

To determine the effect of exposure to cellular free cholesterol on pre-beta HDL, these lipoproteins were incubated for 2 h at 37°C with cholesterol-loaded human fibroblasts. After incubation and recovery of the lipoproteins, the total cholesterol to apoA-I mass ratio was compared with that ratio of pre-beta HDL prior to incubation. The cholesterol content of the pre-beta HDL increased approximately 7-fold after incubation (ranging from 3-fold to 12-fold, **Table 1**), but did not change the electrophoretic mobility of the lipoproteins (**Fig. 5**).

Incubations of alpha HDL with cholesterol-loaded fibroblasts did not alter their electrophoretic mobility or

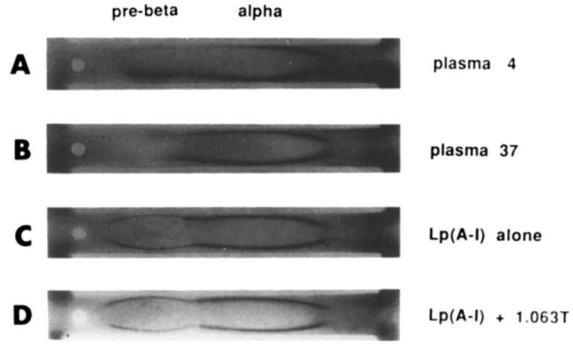

Fig. 4. Incubation of plasma components. Lp(A-I) (100 μg protein) was incubated either alone or with d < 1.063 g/ml lipoproteins (100 μg protein) for 2 h at 37°C and then analyzed by immunoelectrophoresis with antiserum directed against apoA-I. (A) Plasma stored at 4°C; (B) plasma incubated at 37°C; (C) Lp(A-I) incubated at 37°C; (D) Lp(A-I) + d< 1.063 g/ml lipoproteins incubated at 37°C. These findings are representative of the results obtained from three separate experiments.

TABLE 1. Compositional changes in pre-beta HDL after incubation with cholesterol-loaded fibroblasts

Experiment	Protein	Total Cholesterol	TC/Protein	Fold Increase
		mg/ml		
Exp. 1				
Pre-beta HDL before incubation	0.719 ± 0.012	0.004 ± 0.0001	0.005	
Pre-beta HDL after incubation and reisolation[a]	0.216 ± 0.009	0.007 ± 0.0001	0.032	6
Exp. 2				
Pre-beta HDL before incubation	1.78	0.012	0.007	
Pre-beta HDL after incubation and reisolation	0.312	0.0061	0.020	3
Exp. 3				
Pre-beta HDL before incubation	1.080 ± 0.060	0.0028 ± 0.0025	0.003	
Pre-beta HDL after incubation and reisolation	0.088 ± 0.004	0.0033 ± 0.0001	0.037	12

The results of experiments 1 and 3 are the averages and standard deviations of measurements performed in triplicate. The results of experiment 2 are the means of duplicate measurements.

[a]These values represent the concentrations of the reisolated material and do not reflect the recovery of pre-beta HDL.

promote the generation of pre-beta HDL (Fig. 5). The total cholesterol mass of the alpha HDL appeared to increase slightly after exposure to the fibroblasts, significantly increasing its unesterified cholesterol content (data not shown).

Incubation of alpha HDL with cholesteryl ester transfer protein (CETP) and LDL (**Fig. 6** and **Fig. 7**) or VLDL (Fig. 7) for 5 h at 37°C caused a portion of the apoA-I mass to migrate with pre-beta electrophoretic mobility. During analogous incubations involving LDL from patients with Tangier disease, we observed that the cholesteryl ester content of the LDL increased from 21.8% to 33.1% of mass (35), indicating that cholesteryl esters were transferred from HDL to LDL. We verified, by molecular sieve chromatography, that pre-beta HDL-like particles appear to be generated; newly formed particles eluted with the same elution volume as isolated pre-beta HDL (**Fig. 8**). No degradation of apoA-I was detected by SDS-PAGE as a result of the 5-hr incubation (data not shown). We also found that the electrophoretic mobility of apoA-II was not altered by the 5-h incubation (**Fig. 9**), suggesting that the newly formed particles contained no detectable apoA-II similar to pre-beta HDL.

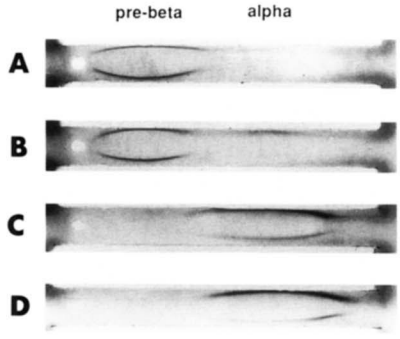

Fig. 5. Incubation of HDL with cholesterol-loaded fibroblasts. Alpha HDL and pre-beta HDL were incubated with cholesterol-loaded human fibroblasts for 2 h at 37°C. After incubation, the lipoproteins were reisolated by selected-affinity immunosorption and subjected to immunoelectrophoresis with antiserum directed against apoA-I. (A) Pre-beta HDL prior to incubation; (B) pre-beta HDL after incubation with fibroblasts; (C) alpha HDL prior to incubation; (D) alpha HDL after incubation with fibroblasts. These findings are representative of the results obtained from three experiments.

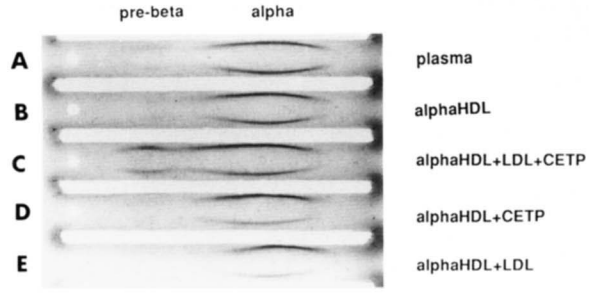

Fig. 6. Incubation of alpha HDL with CETP. Alpha HDL (100 μg protein) were incubated with CETP (8 μg) and LDL (100 μg protein) for 5 h at 37°C and analyzed by immunoelectrophoresis with antiserum directed against apoA-I. (A) Plasma stored at 4°C; (B) isolated alpha HDL incubated at 37°C; (C) alpha HDL incubated with CETP and LDL; (D) alpha HDL incubated with CETP; (E) alpha HDL incubated with LDL. These findings are representative of the results obtained from three separate experiments.

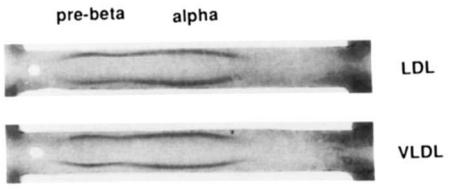

Fig. 7. Incubation of alpha HDL with CETP and various acceptor lipoproteins. Alpha HDL (100 μg protein) were incubated with CETP (8 μg) and either LDL (100 μg, top) or VLDL (100 μg, bottom) for 5 h at 37°C. After incubation the two samples were analyzed by immunoelectrophoresis with antiserum directed against apoA-I.

Insufficient amounts of the pre-beta HDL-like particles were generated to obtain a complete compositional analysis; therefore, complete identity with pre-beta HDL isolated from plasma could not be accomplished.

All three components, alpha HDL, CETP, and LDL, appeared to be necessary for the production of pre-beta HDL. Pre-beta HDL could not be generated by the incubation of alpha HDL with LDL alone or by the incubation of alpha HDL with CETP alone (Fig. 6). These findings indicate that the generation of pre-beta HDL-like particles was not due to the CETP-facilitated rearrangement of HDL.

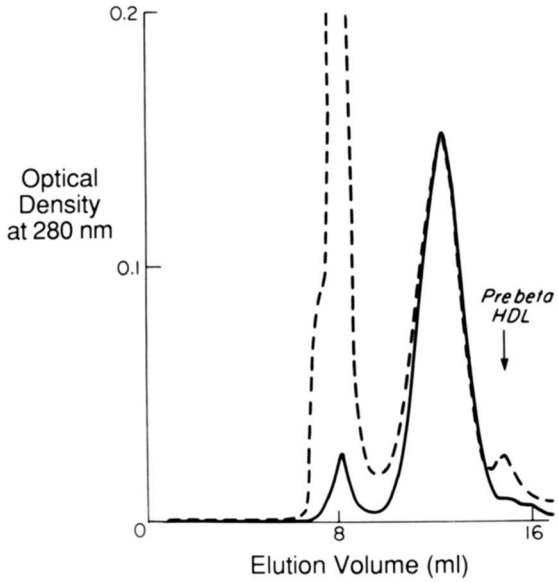

Fig. 8. Molecular sieve chromatography of incubated alpha HDL. The alpha HDL samples incubated with CETP and LDL (shown in Fig. 6), were analyzed by molecular sieve chromatography with a Superose 12 column. The elution profile(OD 280 nm) of the isolated alpha HDL(———) is compared with the elution profile of the incubated alpha HDL+CETP+LDL mixture (---). The elution volume of isolated pre-beta HDL is indicated by the arrow. The peaks having retention volume of approximately 8 ml represent those particles eluting in the void volume; in the case of the alpha HDL, it represents a few large apoA-I-containing lipoproteins, and in the case of the mixture, it represents mostly the LDL.

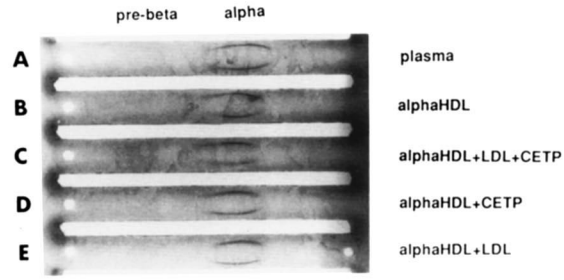

Fig. 9. Incubation of alpha HDL with CETP. Alpha HDL (100 μg) were incubated with CETP (8 μg) and LDL (100 μg) for 5 h at 37°C and analyzed by immunoelectrophoresis with antiserum directed against apoA-II. (A) Plasma stored at 4°C; (B) isolated alpha HDL incubated at 37°C; (C) alpha HDL incubated with CETP and LDL; (D) alpha HDL incubated with CETP; (E) alpha HDL incubated with LDL.

DISCUSSION

In this study we found that pre-beta HDL and alpha HDL undergo interconversion, and that this interconversion appears to be associated with the movement of cholesteryl esters into and out of the HDL compartment. Specifically, we observed that pre-beta HDL disappear from plasma by a process that apparently involves LCAT, and that pre-beta HDL-like particles are generated from alpha HDL by a process that seems to involve CETP and acceptor lipoproteins.

The movement of apoA-I from pre-beta HDL to alpha HDL that we observed appears to be linked to LCAT activity. Two different inhibitors of LCAT, DTNB and menthol, blocked the disappearance of pre-beta HDL from incubated plasma. As the mode of action of these two inhibitors appears to be distinct (36, 37), it is unlikely that anything other than the inhibition of LCAT activity (such as a nonspecific reaction with sulfhydryl groups) was responsible for blocking the conversion of pre-beta HDL. We have no evidence as to the mechanism by which pre-beta HDL apoA-I mass converts to alpha electrophoretic mobility, but two possibilities exist. Either pre-beta HDL themselves could enlarge and undergo structural rearrangements to become particles with alpha electrophoretic mobility or pre-beta HDL could fuse with existing alpha HDL.

The reports of other researchers appear to agree with our findings. Ishida, Albee, and Paigen in mice (7) and Neary et al. in humans (38) have reported on disappearance of pre-beta HDL from incubated plasma. The movement of cell-derived cholesterol from pre-beta HDL to alpha HDL has been observed by Castro and Fielding (18) and Francone, Gurakar, and Fielding (19).

Increases in the apparent diameters of apoA-I-containing lipoproteins when plasma was incubated at 37°C have been observed (39–43). Furthermore, size increases in HDL_3 (44) and in reconstituted HDL (45–49)

have been observed after incubation of these particles with LCAT. Although a change in the size of HDL does not necessarily equate with a corresponding change in electrophoretic mobility, the observed size increases are consistent with our observation on the conversion of pre-beta HDL to alpha HDL following incubation.

To determine whether exposure of either pre-beta HDL or alpha HDL to cellular free cholesterol would alter their respective electrophoretic mobilities, we incubated each HDL subfraction with cholesterol-loaded human fibroblasts. We observed no change in the electrophoretic mobility of either subfraction, indicating that exposure to cellular free cholesterol did not cause transformation of pre-beta HDL into alpha HDL or generation of pre-beta HDL from alpha HDL, despite the apparent acceptance of cellular free cholesterol by these particles.

The concentration of pre-beta HDL-like particles relative to alpha HDL has been found to be elevated in the lymph of dogs (9) and humans (50) compared to their relative concentrations in plasma. If this observed enrichment of pre-beta HDL in lymph is due to the remodeling of HDL rather than to the preferential filtration of small particles through the interstitium, then our findings suggest that other events, in addition to the exposure to cellular free cholesterol, occur in the periphery that cause the generation of pre-beta HDL. The acceptance of cellular cholesterol by pre-beta HDL is consistent with the findings that pre-beta HDL may be the initial acceptors of cellular free cholesterol in plasma, and does not exclude the possibility that larger forms of pre-beta migrating apoA-I-containing lipoproteins could be generated by the acceptance of free cholesterol (18, 19).

Pre-beta HDL-like particles appear to be generated by incubating alpha HDL with CETP and VLDL or LDL. These particles were pre-beta HDL-like in that apoA-I was found in the pre-beta mobility zone, no apoA-II was detected in this zone, and particles generated by the incubation were the same size as isolated pre-beta HDL. Pre-beta HDL-like particles could only be generated by incubation of all three components together. The incubation of alpha HDL with either CETP or acceptor lipoproteins (LDL or VLDL) alone did not produce pre-beta HDL-like particles, indicating that this is not simply a rearrangement of HDL. This finding suggests that pre-beta HDL are formed from alpha HDL as a consequence of the transfer of cholesteryl esters from alpha HDL to acceptor lipoproteins. The generation of pre-beta HDL-like particles could occur by two possible mechanisms. Either pre-beta HDL-like particles could be formed by the dissociation of apoA-I from alpha HDL as the content of their core lipids decreased or lipids could be selectively removed from a specific alpha HDL subfraction that contains apoA-I without apoA-II.

The production of small HDL as a consequence of incubation of HDL with acceptor lipoproteins and CETP has been documented (51–54). However, the CETP-facilitated production of pre-beta HDL that we observed has not been described previously.

Taken together, all of these findings can be assembled into a simple model that describes the relationship between pre-beta HDL and alpha HDL linked to the passage of cholesteryl esters through the total pool of plasma HDL (HDL compartment, **Fig. 10**). In this model apoA-I cycles between pre-beta HDL and alpha HDL, and this

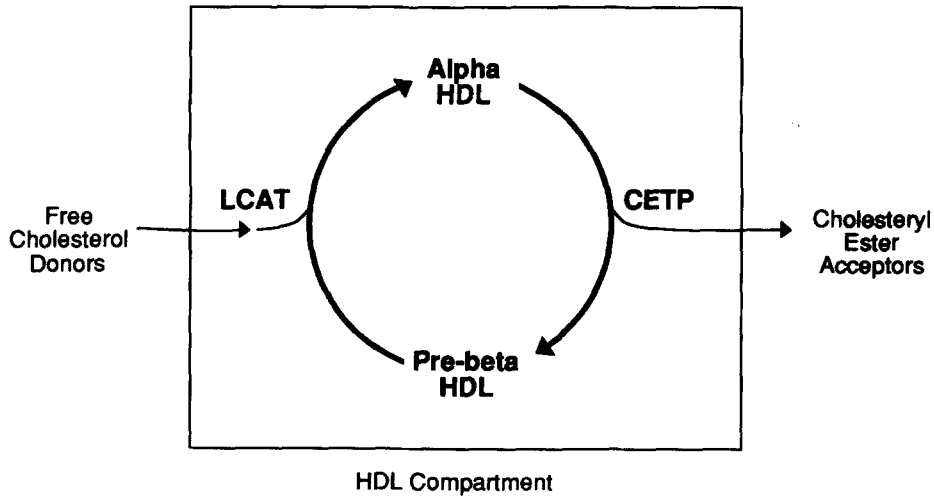

Fig. 10. Proposed model for the interconversion of pre-beta HDL and alpha HDL. ApoA-I cycles between pre-beta HDL and alpha HDL in response to the movement of cholesteryl esters through the HDL compartment. Adding cholesteryl esters to the HDL compartment (by LCAT-catalyzed esterification of free cholesterol) increases the proportion of alpha HDL, whereas removing cholesteryl esters from the HDL compartment (by the CETP-mediated transfer to VLDL and LDL) increases the proportion of pre-beta HDL.

cycling is coupled to the action of LCAT and CETP. As free cholesterol, derived from donors, enters the HDL and is esterified by LCAT, pre-beta HDL are converted into alpha HDL. Conversely, when cholesteryl esters that have accumulated in the HDL are transferred to acceptor lipoproteins (VLDL or LDL) by the action of CETP, pre-beta HDL are generated from alpha HDL.

This model can be used to make specific predictions about the relative concentrations of pre-beta HDL and alpha HDL in human plasma. In cases where the rate of accumulation of cholesteryl esters within the HDL compartment (LCAT activity) is greater than the rate of their transfer out (CETP activity), the relative amount of alpha HDL is expected to increase. Conversely, when transfer of cholesteryl esters out of the HDL compartment outpaces their accumulation, the relative amount of pre-beta HDL is expected to increase. This model is consistent with the high concentration of pre-beta HDL-like particles found in LCAT deficiency (where cholesteryl esters are not supplied to HDL via plasma esterification) (10, 11) and in galactosamine-treated rats (where LCAT activity is diminished) (55). It is also consistent with the positive correlation between the plasma concentrations of pre-beta HDL and triglycerides (14–16); a large number of acceptor lipoproteins would be expected to increase the rate of transfer of cholesteryl esters out of the HDL and thereby increase the relative concentration of pre-beta HDL. While yet untested, we predict that the relative plasma concentration of pre-beta HDL is decreased in patients with CETP deficiency where transfer of cholesteryl esters from HDL to lipoprotein acceptors is blocked.

Another example of the dynamic action of this model is our short (2 h) incubation of normolipidemic fasting plasma. Pre-beta HDL probably disappears from plasma because the rate of cholesterol esterification by LCAT is more rapid than the rate of CETP-facilitated cholesteryl ester transfer (56, 57). Pre-beta HDL reappears during longer incubations (7), when esterification of free cholesterol slows due to depletion of substrate but transfer of cholesteryl esters continues. This consideration underscores the fact that the rate and extent to which the pre-beta HDL disappear from any individual plasma sample are expected to depend upon the absolute concentration of pre-beta HDL and the relative rates of esterification and transfer in that sample.

The model presented here relates the interconversion of pre-beta HDL and alpha HDL to cholesterol esterification and transfer. It should be emphasized, however, that this model is not meant to be exclusive. Other metabolic events that have been shown to alter HDL particle size, such as the action of lipases on lipoproteins (58, 59), the selective uptake of HDL cholesteryl esters (60–62), and the retroendocytosis of HDL (63–66), could also participate in the interconversion between pre-beta HDL and alpha HDL.

This research was supported by NIH grants HL-31210 and HL-14237 (Arteriosclerosis SCOR). We wish to thank Dr. J. P. Kane for his advice and support. We also thank Dr. P. Hass, Genentech, for generously supplying the CETP.

Manuscript received 23 March 1992 and in revised form 30 June 1992.

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