Formation of Spherical, Reconstituted High Density Lipoproteins Containing Both Apolipoproteins A-I and A-II Is Mediated by Lecithin:Cholesterol Acyltransferase*

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Previous studies have provided detailed information on the formation of spherical high density lipoproteins (HDL) containing apolipoprotein (apo) A-I but no apoA-II (A-I HDL) by an lecithin:cholesterol acyltransferase (LCAT)-mediated process. In this study we have investigated the formation of spherical HDL containing both apoA-I and apoA-II (A-I/II HDL). Incubations were carried out containing discoidal A-I reconstituted HDL (rHDL), discoidal A-II rHDL, and low density lipoproteins in the absence or presence of LCAT. After the incubation, the rHDL were reisolated and subjected to immunoaffinity chromatography to determine whether A-I/II-HDL were formed. In the absence of LCAT, the majority of the rHDL remained as either A-I rHDL or A-II rHDL, with only a small amount of A-I/II rHDL present. By contrast, when LCAT was present, a substantial proportion of the reisolated rHDL were A-I/II rHDL. The identity of the particles was confirmed using apoA-I rocket electrophoresis. The formation of the A-I/II rHDL was influenced by the relative concentrations of the precursor discoidal A-I and A-II rHDL. The A-I/II rHDL included several populations of HDL-sized particles; the predominant population having a Stokes' diameter of 9.9 nm. The particles were spherical in shape and had an electrophoretic mobility slightly slower than that of the α-migrating HDL in human plasma. The apoA-I/apoA-II molar ratio of the A-I/II rHDL was 0.7:1. Their major lipid constituents were phospholipids, unesterified cholesterol, and cholesteryl esters. The results presented are consistent with LCAT promoting fusion of the A-I rHDL and A-II rHDL to form spherical A-I/II rHDL. We suggest that this process may be an important source of A-I/II HDL in human plasma.

The high density lipoproteins (HDL) in human plasma comprise several subpopulations of particles with different composition, particle size, and anti-atherogenic potential (1). HDL contain two main apolipoproteins: apolipoprotein (apo)A-I and apoA-II, which account for 70 and 20%, respectively, of HDL protein. These apolipoproteins define two apolipoprotein-specific HDL subpopulations (2): A-I HDL, which contain apoA-I but no apoA-II, and A-I/II HDL, which contain both apoA-I and apoA-II. The presence of apoA-II in HDL reduces their ability to protect against atherosclerosis (3–5).

ApoA-I is synthesized in the liver and intestine and secreted into plasma mainly as discoidal particles containing apoA-I complexed with phospholipids (6, 7). Discoidal A-I HDL are also formed extracellularly by the interaction of lipid-poor apoA-I with phospholipids and unesterified cholesterol from other plasma lipoproteins or cell membranes (8–10). Lipid-poor apoA-I is generated within the plasma as a product of the remodeling of HDL by plasma factors such as cholesteryl ester transfer protein (11, 12), phospholipid transfer protein (13), and hepatic lipase (14). Once formed, discoidal A-I HDL are excellent substrates for lecithin:cholesterol acyltransferase (LCAT), which rapidly esterifies their cholesterol (15). The cholesteryl esters that are formed partition into the center of the particles in a process that converts the disc into spherical A-I HDL (16). LCAT also promotes the fusion of A-I HDL particles in a process that increases the number of apoA-I molecules/particle (17).

The mechanism by which apoA-II becomes a component of spherical A-I/II HDL is not known. ApoA-II is secreted from the liver into the plasma either as discoidal A-II HDL (18) or in a lipid-poor form (19), where it acquires phospholipids from cell membranes in a process that forms discoidal particles (9). However, unlike discoidal A-I HDL, discoidal A-II HDL are nonreactive with LCAT (9, 20) and are therefore not converted into spherical particles. In this paper, we show that spherical A-I/II reconstituted HDL (rHDL) can be assembled from discoidal A-I rHDL and discoidal A-II rHDL in a fusion process promoted by LCAT. This finding represents the first demonstration of a physiological mechanism by which spherical A-I/II HDL may be formed. It also provides a new model with which to investigate how apoA-II impacts on the structure, function, and metabolism of HDL.

EXPERIMENTAL PROCEDURES

Isolation of Lipoproteins and Apolipoproteins

Plasma was obtained from normal volunteers who had fasted for 12 h. HDL (1.063 < d < 1.21 g/ml) and LDL (1.019 < d < 1.055 g/ml) were isolated from pooled human plasma (Transfusion Service, Royal Adelaide Hospital, Australia) by sequential ultracentrifugation in a Beckman L8–70 m ultracentrifuge (21). Human apoA-I and apoA-II were purified to homogeneity following delipidation of HDL (22) and...
chromatography on a column of Q Sepharose Fast Flow (Amersham Pharmacia Biotech) (23). Lipoproteins and lipid-free apolipoproteins were dialyzed against Tris-buffered saline (0.01 M Tris buffer (pH 7.4) containing 0.15 M NaCl, 0.01% (w/v) EDTA-Na₂, and 0.02% (w/v) Na₃PO₄) prior to use in incubations.

Isolation and Assay of LCAT

LCAT was isolated from pooled human plasma as described previously (24). Activity of the preparations was determined using discoidal rHDL prelabeled with [³H]cholesterol (25). The activity of each LCAT preparation is included in the legends to the Figures and Tables.

Preparation of Discoidal rHDL

Discoidal rHDL were prepared by the cholate dialysis method (26) from 1-palmitoyl-2-oleyl phosphatidylcholine (Sigma), unesterified cholesterol (Sigma), and either apoA-I or apoA-II. The final molar ratio (1-palmitoyl-2-oleyl phosphatidylcholine:unesterified cholesterol:protein) of the particles was 68:5:1 and 72:5:1 for the A-I rHDL and the A-II rHDL, respectively. Discoidal A-I rHDL and A-II rHDL made according to this method have 2 molecules of apoA-I and 4 molecules of apoA-II/particle, respectively (23, 27). The rHDL were dialyzed against 5 × 1 liter Tris-buffered saline prior to use in incubations.

Experimental Conditions and Processing of Samples

Various combinations of discoidal A-I rHDL, discoidal A-II rHDL, LCAT, LDL, bovine serum albumin (BSA; Sigma), and β-mercaptoethanol (Sigma) were incubated at 37 °C in sealed tubes for 24 h in a shaking water bath. Details of the individual incubations are described in the legends to the Figures and Tables.

All incubations were terminated by placing the tubes on ice. After the incubations, the HDL density fraction of 1.1–1.25 g/ml was isolated by sequential ultracentrifugation with two 16-h spins at 100,000 rpm at the lower density and a single 16-h spin at 100,000 rpm at the higher density. A Beckman 100.4 Ti rotor and a Beckman TL-100 Tabletop ultracentrifuge maintained at 4 °C were used for these procedures. Aliquots of the HDL density fraction were subjected to immunoaffinity chromatography to isolate A-I/A-II HDL. The reisolated rHDL were then characterized by nondenaturing gradient gel electrophoresis, immunoblot analysis, agarose gel electrophoresis, apoA-I rocket electrophoresis, and electron microscopy and assayed for lipids and apolipoproteins as described below.

Imunoaffinity Chromatography

Raising of Antiserum to apoA-I and apoA-II—Antiserum to purified human apoA-I and apoA-II were raised in sheep and purified by affinity chromatography as described previously (28). ApoA-I antibody preparations were monospecific to human apoA-I, and apoA-II antibody preparations were monospecific to human apoA-II, as judged by immunoblots against purified human apoA-I, apoA-II, and albumin.

Preparation of Anti-apoA-I and Anti-apoA-II Immunoaffinity Columns—Human apoA-I and human apoA-II antibody preparations were covalently coupled to CNBr-activated Sepharose 4B at a ratio of 1:1 (v:v) according to the manufacturer’s instructions (Amersham Pharmacia Biotech). The capacity of the anti-apoA-I-Sepharose was 200–300 μg of apoA-I/ml of gel, and the capacity of the anti-apoA-II-Sepharose was 110–130 μg of apoA-II/ml of gel.

Isolation of Apolipoprotein-specific HDL by Immunoaffinity Chromatography—Aliquots of the HDL density fraction, which had been isolated from the incubations, were rotated with either anti-apoA-I-Sepharose or anti-apoA-II-Sepharose for 1 h at room temperature in sealed Polypropylene chromatography columns. HDL particles that did not bind to the columns were washed off with Tris-buffered saline (8 column volumes). The column was then washed with 6 column volumes of 0.1 M acetic acid (pH 2.7). HDL particles that had bound to the column generally eluted in 1.5–2 column volumes of acetic acid. They were neutralized immediately with 1 M Tris (pH 11.0). The final concentration of Tris in the fractions was 0.09 M.

Electrophoretic Analysis

ApoA-I Rocket Electrophoresis—Aliquots of rHDL were subjected to apoA-I rocket electrophoresis using commercially available LpAI Hydrazides, which contain antibodies to human apoA-I and human apoA-II (Sebia, Issy-les-Moulineaux, France). This is an established analytical technique that separates HDL containing only apoA-I (and no apoA-II) from those containing apoA-II (including A-I/II HDL and A-II/II HDL) (29). The gels were run according to manufacturer’s instructions and stained with acid violet solution.

Gradient Gel Electrophoresis—Aliquots of the A-I/II HDL were electrophoresed for 3000 V/h on 3–40% nondenaturing polyacrylamide gradient gels, stained with Coomassie Blue G-250, and destained with acetic acid (14). Gels were prepared according to the method of Rainwater et al. (30). The Stokes’ diameter of the particles was calculated with reference to standards in a high molecular mass electrophoresis calibration kit (Amersham Pharmacia Biotech).

In some experiments, duplicate samples of A-I/II HDL were electrophoresed on 3–40% nondenaturing gradient gels and transferred to nitrocellulose membranes (8). To compare the distribution of apoA-I and apoA-II in the isolated particles, half of the membrane was immunoblotted for apoA-I and a duplicate half was immunoblotted for apoA-II (28).

Agarose Gel Electrophoresis—Aliquots isolated from A-I/II HDL were subjected to agarose gel electrophoresis as described previously (28). Each gel included one track of purified lipid-free apoA-I as a marker of pre-β migrating particles and one track of HDL (density fraction 1.065–1.21 g/ml) isolated from human plasma as a marker of α-sedimenting particles.

Electron Microscopy

Electron microscopy of the isolated A-I/II HDL was performed as described previously (31).

Chemical Analyses

All assays were performed on a Cobas-Fara centrifugal analyzer (Roche Diagnostics, Zurich, Switzerland). Concentrations of total cholesterol, unesterified cholesterol, and phospholipids were measured using enzymatic kits (Roche Molecular Biochemicals). The concentration of cholesteryl esters was calculated as the difference between the concentrations of total and unesterified cholesterol. Concentrations of apoA-I and apoA-II were measured immunoturbidometrically as described previously (28), using antisera raised in sheep to human apoA-I (described above) or apoA-II (Roche Molecular Biochemicals). The assay was standardized using appropriate dilutions of either lipid-free apoA-I or apoA-II purified from human plasma as described above.

RESULTS

Role of LCAT in the Formation of A-I/II HDL (Table I)

A mixture of discoidal A-I rHDL and discoidal A-II rHDL was supplemented with LDL (as a source of unesterified cholesterol for the LCAT reaction), BSA, and β-mercaptoethanol and incubated at 37 °C for 24 h in the absence or presence of LCAT. After the incubation, the HDL (1.1–1.25 g/ml) were reisolated by ultracentrifugation. To determine whether A-I/II rHDL particles were formed during the incubations, the HDL were subjected to immunoaffinity chromatography on either an anti-apoA-I-Sepharose column or an anti-apoA-II-Sepharose column. To confirm the integrity of the columns, we showed quantitative binding of A-I HDL to the anti-apoA-I column and A-II HDL to the anti-apoA-II column (results not shown). Conversely, A-II rHDL did not bind to the anti-apoA-I column, and A-I rHDL did not bind to the anti-apoA-II column (results not shown). Incubation of A-I HDL or A-II HDL alone with LCAT had no effect on the binding of either preparation to the anti-apoA-I or the anti-apoA-II column, respectively (results not shown). Thus, any apoA-II that bound to the anti-apoA-I column and any apoA-I that bound to the anti-apoA-II column must, by definition, have been accommodated in A-I/II rHDL particles.

In incubations conducted in the absence of LCAT, 12.5–20% of the apoA-II in the reisolated rHDL mixture bound to the anti-apoA-I-Sepharose column and 0–14% of the apoA-I bound to the anti-apoA-II-Sepharose column (Table I). Thus, in the absence of LCAT, the majority of the particles remaining as either A-I rHDL or A-II rHDL had only a small amount of A-I/II particles present. In contrast, when the incubations contained LCAT, 31–93% of the apoA-II in the reisolated rHDL bound to the anti-apoA-I-Sepharose column, and 51–64% of the apoA-I bound to the anti-apoA-II-Sepharose column (Table I). Thus, in the presence of LCAT, a substantial proportion of the reisolated rHDL were A-I/II rHDL.
Formation of A-I/A-II rHDL

Table I
Formulation of A-I/A-II rHDL in the absence or presence of LCAT

| Incubation mixture       | Reisolated rHDL loaded on anti-apoA-I-Sepharose column (μg of apoA-I/ml) | Reisolated rHDL bound to anti-apoA-I-Sepharose column (% of apoA-I or apoA-II loaded) | Height of rockets |
|--------------------------|--------------------------------------------------------------------------|----------------------------------------------------------------------------------------|------------------|
| Discoidal A-I rHDL       | ApoA-I 795 195                                                        | 100 100                                                                                 | 13.9 3.4         |
| Discoidal A-II rHDL      | ApoA-I 653 306                                                       | 100 100                                                                                 | 7.4 3.1          |
|                          | ApoA-II 702 516                                                      | 100 69                                                                                  | 12.4 6.5         |

Effect of Varying the Relative Concentrations of Discoidal rHDL on the Formation of A-I/A-II rHDL

Increasing Concentrations of Discoidal A-I rHDL, Constant Concentration of Discoidal A-II rHDL (Table II)—The results described above showed that LCAT promoted the formation of A-I/A-II rHDL from precursor discoidal A-I and A-II rHDL. Given that these experiments were carried out at a single concentration of the precursor particles, we next asked whether the formation of A-I/A-II rHDL was influenced by differences in the relative concentrations of the A-I and A-II discoidal rHDL. Studies were conducted with mixtures containing varying proportions of the discoidal particles. A constant amount of discoidal A-I rHDL was incubated with LDL and LCAT for 24 h at 37 °C in the presence of increasing concentrations of discoidal A-II rHDL. Following the incubation, the rHDL were reisolated and subjected to immunoaffinity chromatography on an anti-apoA-I-Sepharose column. In each case, virtually all of the apoA-I in the reisolated rHDL fractions bound to the column (Table II), as indicated by the absence of any measurable apoA-I in the unbound fraction. As the concentration of discoidal A-II rHDL increased, there was an increase in the amount of apoA-I that bound to the anti-apoA-I column, consistent with an increase in the amount of A-I/A-II rHDL formed. At the lower concentrations of apoA-II, all of the apoA-II in the reisolated rHDL also bound to the anti-apoA-I column showing that, under these conditions, all of the apoA-II resided in A-I/A-II rHDL. At higher concentrations of discoidal A-II rHDL, a proportion of the apoA-II did not bind to the column, indicating that it remained as a component of A-II rHDL. This finding may, however, reflect the high amounts of precursor discoidal rHDL present. Under these conditions, the amount of LCAT present may not have been sufficient to promote the fusion of all of the discoidal A-II rHDL with A-I rHDL. Any A-II rHDL that remained would not have bound to the anti-apoA-I column.

Binding of rHDL to an anti-apoA-I-Sepharose column indicates the presence of apoA-I in the particle, regardless of whether the apoA-I is in A-I rHDL or A-I/A-II rHDL. To confirm that both A-I rHDL and A-I/A-II rHDL were present, the bound rHDL were eluted from the column and subjected to apoA-I rocket electrophoresis (Table II). This is an established technique used to detect A-I rHDL and A-I/A-II rHDL in human serum (29). Human HDL generates two rockets; the height of the higher, fainter rocket is proportional to the amount of A-I rHDL and the lower, darker rocket corresponds to rHDL particles containing apoA-II (including A-I/A-II HDL and A-II HDL) (29). In the experiments shown in Table II, A-I rHDL and A-I/A-II rHDL were detected under all incubation conditions. As the concentration of discoidal A-II rHDL increased, there was evidence of an increase in the proportion of A-I/A-II rHDL relative to A-I rHDL (Table II). This observation is consistent...
with the increasing amount of apoA-II binding to the anti-apoA-I column as described above.

**Increasing Concentrations of Discoidal A-I rHDL, Constant Concentrations of Discoidal A-II rHDL (Table III)—**To determine the effect of increasing the concentration of discoidal A-I rHDL on the formation of A-I/A-II rHDL, mixtures of discoidal A-II rHDL, LDL, and LCAT were incubated for 24 h at 37 °C with increasing concentrations of discoidal A-I rHDL. Follow- ing the incubations, the rHDL were reisolated by ultracentrifugation and subjected to immunoaffinity chromatography on an anti-apoA-I-Sepharose column. Irrespective of the initial concentration of discoidal A-I rHDL, all of the apoA-I and all of the apoA-II in each of the reisolated rHDL fractions bound to the anti-apoA-I column (Table III), as indicated by an absence of apoA-I and apoA-II in the unbound fractions. Thus, under the incubation conditions used in this experiment, all of the apoA-II resided in A-I/A-II rHDL particles. As the concentration of discoidal A-I rHDL increased, there was an increase in the amount of apoA-I that bound to the anti-apoA-I column, whereas the amount of apoA-II that bound to the column remained constant (Table III). This result is consistent with no

**FIG. 1.** ApoA-I rocket electrophoresis of isolated A-I/A-II rHDL.

Discoidal A-I rHDL (final concentration 96 μg of apoA-I/ml of incubation mixture) were incubated with discoidal A-II rHDL (final concentration 29 μg of apoA-II/ml of incubation mixture) at 37 °C for 24 h with LDL (final concentration 500 μg of apoB/ml of incubation mixture), LCAT (210 ml), BSA (3% w/v), and β-mercaptoethanol (final concentration 4.4 mM). The LCAT activity was 167 nmol of cholesterol esterified/h/ml of LCAT. The final incubation volume was 726 ml. After the incubation, the total rHDL fraction was reisolated, and the A-I/A-II rHDL was separated by immunoaffinity chromatography on an anti-apoA-I-Sepharose column. Aliquots of the mixture of A-I/A-II rHDL and A-I rHDL that was reisolated from the incubation (lane A) and the isolated A-I/A-II rHDL (lane B) were subjected to apoA-I rocket electrophoresis as described under “Experimental Procedures.”

**FIG. 2.** Size and apolipoprotein distribution of isolated A-I/A-II rHDL.

Discoidal A-I rHDL were incubated with discoidal A-II rHDL, and A-I/A-II rHDL were isolated as described in the legend to Fig. 1. An aliquot of the isolated A-I/A-II rHDL was electrophoresed on a 3–40% nondenaturing gradient gel as described under “Experimental Procedures.” The gel was stained, and the distribution profile was obtained by laser densitometric scanning (A). Immunoblotting of nondenaturing gradient gels to detect apoA-I and apoA-II was carried out as described under “Experimental Procedures” (B). In B, numbers refer to the Stokes’ diameter of high molecular weight protein standards.
change in the amount of A-I/A-II rHDL, but an increase in the amount of A-I rHDL when increasing concentrations of precursor A-I rHDL particles are present.

Having established that there were no A-II rHDL remaining after the incubation, the rHDL that were reisolated from the incubation mixtures were subjected to apoA-I rocket electrophoresis to determine the relative amounts of A-I rHDL and A-I/A-II rHDL present. Each of the reisolated rHDL fractions yielded two rockets (Table III) indicating the presence of both A-I rHDL and A-I/A-II rHDL under all incubation conditions. However, with increasing concentrations of discoidal A-I rHDL in the initial incubation mixture, the amount of A-I rHDL relative to A-I/A-II rHDL also increased (Table III).

**Isolation and Characterization of A-I/A-II rHDL**

To isolate and characterize the A-I/A-II rHDL formed during incubation of discoidal A-I rHDL, discoidal A-II rHDL, and LCAT, a larger scale incubation was conducted under conditions designed to yield a mixture of A-I rHDL and A-I/A-II rHDL in which no A-II rHDL remained. After the incubation, the rHDL were reisolated by ultracentrifugation. To confirm the absence of A-II rHDL, a small aliquot of the reisolated rHDL was subjected to immunoaffinity chromatography on an anti-apoA-I-Sepharose column. The fact that all the apoA-II bound to the anti-apoA-I column (results not shown) confirmed the absence of A-II rHDL in the reisolated particles. A-I/A-II rHDL were separated from the mixture of A-I rHDL and A-I/A-II rHDL by immunoaffinity chromatography on an anti-apoA-II-Sepharose column. The rHDL that bound to the anti-apoA-II column were eluted from the column and subjected to further characterization. To confirm that the particles that bound to the anti-apoA-II column were solely A-I/A-II rHDL with no A-I rHDL, the particles were subjected to apoA-I rocket electrophoresis (Fig. 1). The total mixture of A-I and A-I/A-II rHDL that was reisolated from the incubation generated both a faint rocket and a dark rocket, consistent with the presence of both A-I rHDL and A-I/A-II rHDL, respectively (Fig. 1A). In contrast, the rHDL, which bound to and eluted from the anti-apoA-II column, generated only a single, dark rocket establishing the presence of A-I rHDL but an absence of A-I rHDL (Fig. 1B).

The size distribution of the isolated A-I/A-II rHDL was determined by nondenaturing gradient gel electrophoresis. The A-I/A-II rHDL included one major population of particles with a Stokes’ diameter of 9.9 nm (Fig. 2A). There were also some less abundant larger and smaller particles present with Stokes’ diameters of 17.0 and 7.8 nm, respectively (Fig. 2A). The distribution of apoA-I and apoA-II in the isolated A-I/A-II rHDL was determined by immunoblot analysis of nondenaturing gradient gels. ApoA-I was equally distributed between the 9.9- and 7.8-nm particles with much less present in the 17.0-nm particles (Fig. 2B). ApoA-II was found predominantly in the 9.9-nm particles with much less in the populations of larger and smaller particles (Fig. 2B).

When subjected to agarose gel electrophoresis (Fig. 3), the A-I/A-II rHDL had a mobility slightly slower than that of the α-migrating HDL in human plasma. They also included a minor population of pre-β-migrating particles.

The lipid and apolipoprotein composition of the isolated A-I/A-II rHDL is shown in Table IV. For comparison, the composition of the precursor discoidal A-I and A-II rHDL and the mixture of A-I rHDL and A-I/A-II rHDL from which the A-I/A-II rHDL were isolated is also shown. The isolated A-I/A-II rHDL contained both apoA-I and apoA-II and had acquired a core of cholesteryl esters when compared with the precursor discoidal particles. The A-I/A-II particles contained similar percentages of phospholipids, unesterified cholesterol, and cholesteryl esters to the total rHDL mixture from which they were isolated. The A-I/A-II rHDL were depleted of apoA-I and enriched in apoA-II, consistent with the fact that the total fraction contained both A-I rHDL and A-I/A-II rHDL. In agreement with this, the apoA-I/apoA-II molar ratio of the isolated A-I/A-II rHDL was 0.7:1, whereas that of the total rHDL mixture was 2.5:1 (results not shown). It should be noted that the apoA-I/apoA-II molar ratio of the A-I/A-II particles does not refer to each single particle of A-I/A-II rHDL but rather reflects the average of several populations of particles of varying size, which contain different proportions of apoA-I and apoA-II (Fig. 2, A and B). When subjected to electron microscopy (Fig. 4), the A-I/A-II particles were found to be spherical in shape and heterogeneous in size with diameters ranging from 8.2 to 14.3 nm (mean 8.8 nm, n = 130).

**Origin of the Cholesteryl Esters in A-I/A-II rHDL**

To determine the origin of the cholesteryl esters in the isolated A-I/A-II rHDL, discoidal A-I and A-II rHDL, which con-
tained unesterified cholesterol labeled with $^{14}$C and $^3$H, respectively, were incubated with LCAT and LDL under conditions which generated a mixture of A-I rHDL and A-I/II rHDL but no A-II rHDL (Figs. 1–4). After the incubation, the rHDL were resolated by ultracentrifugation and subjected to immunoaffinity chromatography on an anti-apoA-II column. The rHDL that bound and were eluted from the column were subjected to thin layer chromatography. More than 95% of the $^{14}$C and $^3$H label was recovered as cholesteryl esters moiety in the A-I/II rHDL particles. This result indicates that the cholesteryl esters in the A-I/II rHDL particles were derived from the unesterified cholesterol in both the precursor discoidal A-I and A-II rHDL particles.

**DISCUSSION**

A major function of LCAT is to generate cholesteryl esters in A-I HDL. These particles originate as discoidal complexes of apoA-I and phospholipids that are either secreted from the liver or intestine (6, 7). They may also be generated extracellularly from chylomicrons undergoing lipolysis or by the recruitment of lipids from cell membranes or other lipoprotein fractions by lipid-poor apoA-I (8–10). As a direct consequence of the LCAT-catalyzed generation of a core of cholesteryl esters, the discoidal A-I HDL are converted into spherical particles. Continued activity of LCAT on the spherical A-I HDL results in the particles increasing in both size and apoA-I content in a process that involves fusion of A-I HDL (17). In the present study, we have found that LCAT also promotes the fusion of A-I rHDL with A-II rHDL to form spherical A-I/II rHDL. Given that all of the ingredients used in these in vitro studies are present in plasma *in vivo*, we suggest that this process of LCAT-mediated fusion may be an important source of the spherical A-I/II HDL that circulate in plasma.

The results presented in this study are consistent with the conclusion that LCAT mediates a fusion of discoidal A-II rHDL with A-I rHDL resulting in the formation of A-I/II rHDL. The fact that the cholesteryl esters in the A-I/II particles were derived from the unesterified cholesterol in both the precursor discoidal particles is also consistent with the proposal that the A-I/II rHDL were formed by a fusion process. However, it must be noted that the well recognized rapid exchange of cholesteryl between lipoprotein fractions would also have contributed to the presence of both labels in the A-I/II particles. The alternate scenario for the formation of the A-I/II particles is that apoA-II transfers from A-II rHDL discs to the spherical A-I rHDL particles. This explanation is, however, highly unlikely for the following reasons. First, if a proportion of the apoA-II transferred from the A-II rHDL discs, there should be evidence of discoidal A-II rHDL remaining after the incubation. This is not compatible with the finding of conditions in which all of the apoA-II was recovered in A-I/II rHDL (Tables II and III). Second, if all of the apoA-II transferred from the A-II rHDL discs to the A-I rHDL spheres, it would leave particles containing phospholipids and unesterified cholesterol but no apolipoprotein. This situation is thermodynamically highly unlikely. Finally, apoA-II has a very high affinity for lipids (32, 33). It is therefore, highly unlikely that apoA-II would have dissociated from a disc in lipid-poor form and transferred to the apoA-I sphere.

Spherical A-I/II HDL account for about half of the apoA-I and almost all of the apoA-II in human plasma (2), with the remainder of the apoA-I being accommodated in A-I HDL. Until now, the mechanism by which apoA-II becomes a component of spherical A-I/II HDL has been unknown. Like discoidal A-I HDL, there is evidence that discoidal A-II HDL are either secreted from the liver (18) or generated extracellularly by the interaction of lipid-poor apoA-II with phospholipids and unesterified cholesterol in cell membranes or other lipoproteins (8, 9). However, in contrast to discoidal A-I HDL, discoidal A-II HDL are not reactive with LCAT (9, 20), possibly explaining why spherical A-II HDL are not a major component of native HDL (34). The present in vitro studies provide a clear demonstration that discoidal A-II rHDL can fuse with A-I rHDL in an LCAT-mediated process, the end-product of which is spherical A-I/II HDL.

![Figure 4: Electron microscopy of isolated A-I/II rHDL](image)

**FIG. 4.** Electron microscopy of isolated A-I/II rHDL. Discoidal A-I rHDL were incubated with discoidal A-II rHDL, and A-I/II rHDL were isolated as described in the legend to Fig. 1. The isolated A-I/II rHDL were subjected to electron microscopy as described under “Experimental Procedures.” The bar marker represents 100 nm.

**FIG. 5.** Hypothetical model by which LCAT promotes the formation of spherical A-I/II HDL. LCAT-catalyzed generation of a core of cholesteryl esters in discoidal A-I HDL generates small, spherical particles, containing the same number of apoA-I molecules as the precursor discoidal particles. Continued interaction of LCAT with the small, spherical A-I HDL generates more cholesteryl esters. The expanding particles can then fuse with discoidal A-I HDL to form a larger, spherical A-I HDL in which the number of molecules of apoA-I is increased. Alternatively, the expanding spherical A-I HDL can fuse with discoidal A-II HDL to form spherical A-I/II HDL.
A-I/A-II rHDL. The possibility that a comparable process may have generated discoidal particles that have no physiological equivalents and provide no insight into the effects of apoA-II on spherical HDLs. In contrast, the spherical particles prepared by the technique used in the present study resemble their native counterparts in size and composition and are ideal tools with which to investigate how apoA-II impacts on the structure and function of HDL and on the interaction of HDL with a range of the plasma enzymes and transfer proteins that remodel and regulate HDL in plasma.

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