Apolipoprotein A-I Structural Modification and the Functionality of Reconstituted High Density Lipoprotein Particles in Cellular Cholesterol Efflux*

(Received for publication, March 29, 1996, and in revised form, June 4, 1996)

Kristin L. Gillotte, W. Sean Davidson‡, Sissel Lund-Katz, George H. Rothblat, and Michael C. Phillips§

From the Department of Biochemistry, Allegheny University of the Health Sciences, Philadelphia, Pennsylvania 19129 and the Department of Biochemistry, College of Medicine at Urbana-Champaign, University of Illinois, Urbana, Illinois 61801

The role of HDL and its major protein constituent, apolipoprotein (apo) A-I, in promoting the removal of excess cholesterol from cultured cells has been well established; however, the mechanisms by which this occurs are not completely understood. To address the effects of apoA-I modification on cellular unesterified (free) cholesterol (FC) efflux, three recombinant human apoA-I deletion mutants and plasma apoA-I were combined with 1-palmityl-2-oleoyl phosphatidylcholine (POPC) and FC to make reconstituted high density lipoprotein (rHDL) discoidal complexes. These particles were characterized structurally and for their efficiency as acceptors of mouse L-cell fibroblast cholesterol. The deletion mutant proteins lacked NH2-terminal (apoA-I (Δ44–126)), central (apoA-I (Δ139–170)), or COOH-terminal (apoA-I (Δ190–243)) domains of apoA-I. The three deletion mutants all displayed lipid-binding abilities and formed discoidal complexes that were similar in major diameter (13.2 ± 1.5 nm) to those formed by human apoA-I when reconstituted at a 100:5:1 (POPC:FC:protein) mole ratio. Gel filtration profiles indicated unreacted protein in the preparation made withapoA-I (Δ190–243), which is consistent with the COOH terminus portion ofapoA-I being an important determinant of lipid binding. Measurements of the percent α-helix content of the proteins, as well as the number of protein molecules per rHDL particle, gave an indication of the arrangement of the deletion mutant proteins in the discoidal complexes. The rHDL particles containing the deletion mutants had more molecules of protein present than particles containing intact apoA-I, to the extent that a similar number of helical segments was incorporated into each of the discoidal species. Comparison of the experimentally determined number of helical segments with an estimate of the available space indicated that the deletion mutant proteins are probably more loosely arranged than apoA-I around the edge of the rHDL. The abilities of the complexes to remove radiolabeled FC were compared in experiments using cultured mouse L-cell fibroblasts. All four discoidal complexes displayed similar abilities to remove FC from the plasma membrane of L-cells when compared at an acceptor concentration of 50 µg of phospholipid/ml. Thus, none of the deletions imposed in this study notably altered the ability of the rHDL particles to participate in cellular FC efflux. These results suggest that efficient apoA-I-mediated FC efflux requires the presence of amphipathic α-helical segments but is not dependent on specific helical segments.

High density lipoproteins (HDL) are a heterogeneous class of particles thought to mediate the flux of unesterified (free) cholesterol (FC) from peripheral cells to the liver in the process of reverse cholesterol transport (1). However, the mechanism directing the incorporation of cell cholesterol into the HDL and the role of HDL’s major protein component, apolipoprotein (apo) A-I, are subjects of controversy. Epidemiological studies (2) as well as experiments involving transgenic animal models (3) have suggested that apoA-I is the major determinant of the ability of HDL species to participate in cholesterol efflux, thus much effort has been expended to elucidate the functional domains of this protein.

It has been demonstrated that the presence of amphipathic helical segments in acceptor particles is necessary for efficient efflux of cholesterol from cells (4). The arrangement or conformation of these segments may be a factor in the ability of acceptor particles to sequester cellular cholesterol. Several groups have utilized epitope-specific apoA-I monoclonal antibodies to define segment(s) of apoA-I that may be crucial for FC efflux (5–8). Banks et al. (5) utilized eight such antibodies and found a region spanning amino acid residues 74–110 to be important for efficient cholesterol efflux. A similar approach was taken by Luchoomun et al. (6) whose experiments indicated that the domain around amino acid 165 of apoA-I is involved in the efflux of cellular cholesterol. Experiments in which genetic apoA-I variants were combined with dimyristoylphosphatidylcholine have further suggested that substitution of a proline residue at amino acid 165 interferes with a conformation that is essential for cholesterol efflux (7). In contrast, a study by Fielding et al. (8) has demonstrated that the amino acid region 137–144 of apoA-I is adjacent to or part of a segment.

1 The abbreviations used are: rHDL, reconstituted HDL; apo, apolipoprotein; apoA-I (Δ139–170), apoA-I deletion mutant lacking residues Glu139-Leu170; apoA-I (Δ190–243), apoA-I deletion mutant lacking residues Ala190-Gln244; apoA-I (Δ44–126), apoA-I deletion mutant lacking residues Leu44-Leu126; apoA-I/FC/POPC, apoA-I/FC/POPC discoidal complex; CD, circular dichroism; EM, electron microscopy; FC, free (unesterified) cholesterol; HDL, high density lipoprotein; LCAT, lecithin-cholesterol acyltransferase; PAGE, polyacrylamide gradient gel electrophoresis; PL, phospholipid; POPC, 1-palmityl-2-oleoyl phosphatidylcholine.

* This work was supported by National Institutes of Health Program Project HL22633, a predoctoral fellowship (to K. L. G.) from the American Heart Association, Southeastern Pennsylvania Affiliate, and a postdoctoral fellowship (to W. S. D.) from the American Heart Association, Illinois Affiliate. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed.

§ To whom correspondence should be addressed.
structural site in the HDL subspecies, pre-β1-HDL, which is effective in promoting the efflux of cellular cholesterol. Another study has attempted to distinguish whether monoclonal antibodies specific to apoA-I are able to inhibit cellular cholesterol efflux from intracellular or plasma membrane pools of cholesterol (9). Two Fab fragments spanning the amino acid region 140–150 were found to inhibit the efflux of intracellular cholesterol but not the cholesterol flux from the plasma membrane. It is apparent that there is marked disagreement about which, if any, domains of apoA-I are critical for cellular cholesterol efflux.

Recently, Holvoet et al. (10) have expressed and purified recombinant mature human apoA-I and various deletion mutants for investigating structure-function relationships with regard to activation of lecithin-cholesterol acyltransferase (LCAT). The three deletion mutants: apoA-I (ΔLeu44-Leu126), apoA-I (ΔGlu139-Leu170), and apoA-I (ΔAla190-Gln243) lack domains thought to be crucial for either phospholipid (PL) binding or LCAT activation. In an attempt to define the domain of apoA-I that is crucial for cellular cholesterol efflux from cells, the present study uses acceptor particles containing these engineered apoA-I molecules reconstituted with 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC) into homogeneous and highly defined discoidal complexes. The variant proteins were successfully combined with POPC and unesterified cholesterol to form reconstituted HDL (rHDL) particles of similar size, which were subsequently characterized with respect to their structure. Cholesterol efflux studies indicate that when rHDL particles are utilized as acceptors of FC, there are no significant differences in the rates of efflux to the discoidal HDL containing either intact apoA-I or the deletion mutant proteins. These data indicate that the deletion of large segments from either the NH2-terminal, central or COOH-terminal regions of the apoA-I molecule does not significantly impair the functionality of the rHDL complexes as acceptors of cellular cholesterol.

**EXPERIMENTAL PROCEDURES**

**Materials**

1-Palmitoyl-2-oleoyl phosphatidylcholine (POPC) was purchased from Avanti Polar Lipids (Birmingham, AL) (+99% grade). Bovine serum albumin, sodium cholate, gentamycin, and cholesterol methyl ether were obtained from Sigma (St. Louis, MO). [2-3H]Cholesterol was purchased from DuPont NEN (Boston, MA). Bovine serum and minimal essential medium were obtained from Life Technologies, Inc. (Grand Island, NY).

**Methods**

**Purification of Apolipoprotein A-I**—Human HDL isolated from the fresh plasma of normolipidemic subjects was delipidated in ethanol/diethyl ether as described by Scanu and Edelstein (11) and purified apoA-I was isolated by anion exchange chromatography on Q-Sepharose and stored in lyophilized form at −70°C (12). Prior to use the purified apoA-I was resolubilized in 6 M guanidine HCl and dialyzed extensively against Tris buffer (10 mM Tris, 150 mM NaCl, 1.0 mM EDTA; pH 8.2).

**Recombinant Human Apolipoprotein A-I and Deletion Mutations**—Preparation of the recombinant proteins was carried out by Holvoet et al. as formerly described in detail (10). Wild-type apoA-I and the apoA-I mutants were expressed in the periplasmic space of *Escherichia coli* cells and purified to homogeneity to yield the three deletion mutants utilized in this study: apoA-I (ΔLeu44-Leu126), apoA-I (ΔGlu139-Leu170), and apoA-I (ΔAla190-Gln243).

**Preparation of Reconstituted HDL (rHDL)**—Particles were prepared using the cholate dispersion/Bezo-Bead removal technique as described in detail previously (13). A starting POPC/cholesterol/protein ratio of 100:5:1 (mol:mol: mol) was used and final compositions were determined after concentration (Centriprep 30, Amicon) under low speed centrifugation. The homogeneity and size of the complexes were assessed by gradient gel electrophoresis using precast 8–25% polyacrylamide gels (Pharmacia Biotech Inc.). Due to indications that the complex preparations contained varying amounts of free protein, the samples were purified by high performance gel filtration (Superdex 200 HR 10/30, Pharmacia Biotech Inc.). The column was calibrated with standard proteins (13) and the hydrodynamic diameters of the particles were calculated from the elution volumes.

**Characterization of rHDL**—The particles were analyzed chemically using the Markwell modification of the Lowry protein assay (14), while phospholipids were determined as inorganic phosphorus by the method of Sokoloff and Rothblat (15). Unesterified cholesterol was determined after Bligh and Dyer extraction (16) by gas-liquid chromatography analysis (17); cholesteryl methyl ether was utilized as an internal standard in this assay. The number of apoA-I molecules per particle was determined by reaction with dimethyl suberimidate and determining the degree of cross-linking of the apoA-I by SDS-PAGE (18). In addition to hydrodynamic diameter determination by gel filtration chromatography, negative stain electron microscopy (EM) was utilized as described for 18.9 μl of rHDL were analyzed in a JEM 1230 electron microscope (Hitachi, Japan). A minimum of 100 particles were analyzed for each sample.

**Efflux of Plasma Membrane Cholesterol**—Mouse L-cell fibroblast monolayers were used to monitor the release of [3H]cholesterol to the extracellular medium as a measure of unesterified (free) cholesterol (FC) efflux, as has been described in detail (4). The cells, present in 12-well cell plates (22 mm), were grown to confluence in minimal essential medium/bicarbonate supplemented with 10% fetal bovine serum in a 37°C humidified incubator in the presence of 95% air and 5% CO2. The monolayers were labeled with 2 μCi/ml (1.25×105 cpm) [3H]cholesterol in bicarbonate-buffered minimal essential medium with 2.5% fetal calf serum for 24 h. This was followed by a 12-h incubation in minimal essential medium/bicarbonate containing 1% bovine serum albumin to equilibrate the radioactivity between the various cellular sterol pools. After a brief wash with minimal essential medium/bicarbonate containing 1% bovine serum albumin and the acceptor at the indicated PL concentration, the experiment was conducted in a 37°C incubator with an atmosphere of 95% air and 5% CO2. The radioactivity of a 75-μl aliquot of the medium was determined at specific time intervals to estimate the fraction of FC released into the medium. Upon completion of the time course, all cell wells were washed with Dulbecco’s phosphate-buffered salt solution three times and the cellular lipids were extracted with isopropanol (21). From the extraction, the total amount of radioactive cholesterol per well was determined by liquid scintillation counting.

**Results**

**Structural Characterization of rHDL Particles**—Previously, plasma apoA-I has been shown to form discoidal complexes (rHDL) upon combination with POPC + cholesterol (22–24), and the compositional characteristics of the discoids have been well defined. Holvoet et al. (10) recently showed that recombinant apoA-I and specific deletion mutants of apoA-I were able to combine in a similar manner with dipalmitoylphosphatidy-
choline to form discoidal complexes. We confirmed that recombinant apoA-I forms reconstituted complexes with POPC and cholesterol and that these complexes are structurally identical to those prepared from plasma apoA-I. In preliminary cell FC efflux studies utilizing rHDL containing either plasma apoA-I or recombinant, wild-type, apoA-I, all measurements of efflux to the two types of rHDL particles were indistinguishable, indicating that the origin of the apoA-I did not affect the ability of the particles to function as acceptors of cellular cholesterol. Therefore, plasma apoA-I was used as the control protein in the studies of recombinant variants.

To study the effects of apoA-I modification on the structure and function of rHDL particles, discoidal complexes were prepared with plasma apoA-I, apoA-I (Δ144–126), apoA-I (Δ139–170), or apoA-I (Δ190–243) at an initial molar ratio of 100:5:1 (POPC:FC:protein). The particles were sized by PAGE, which indicated the presence of free protein in addition to the complexes, particularly with rHDL constructed with apoA-I (Δ44–126) and apoA-I (Δ190–243) (approximately 20 and 50% lipid-free protein, respectively). The complexes were isolated from unreacted protein and lipid using gel filtration chromatography on a calibrated column, from which particle hydrodynamic diameters were determined. The gel filtration profiles (Fig. 1) indicated that there was no free protein in the preparations containing plasma apoA-I, apoA-I (Δ44–126), and apoA-I (Δ139–170), whereas 26% of apoA-I (Δ190–243) was found to be unassociated with lipid in its preparation. These results suggest that the extent of free protein detected by PAGE may have been an artifact of the electrophoresis due to stripping of the protein from the complexes as they migrated through the gel. Reduced lipid association of apoA-I (Δ190–243) was an expected result as this apoAI mutant is missing the carboxyl terminus of apoA-I, which plays a major role in stabilizing the lipid-bound state of apoA-I (10, 25–27). The fractions corresponding to the rHDL species were isolated and characterized; the compositions and dimensions of the particles are listed in Table I. Electron microscopy was utilized to confirm that the particles were discoidal in shape, which is apparent from the stacks of discoidal complexes displayed for each preparation in Fig. 2. The composition and size determinations of the plasma apoA-I:FC:POPC complex are in good agreement with previously published results for a discoidal complex of composition 83:3:1 (molar ratio, POPC:FC:apoA-I) (24). This complex has been measured by PAGE and EM to have a diameter of 10.3 and 11.5 nm, respectively, which is the same as the present measurements of 10.0 and 11.2 nm (Table I). The EM measurements denote an average major diameter of 13.2 ± 1.5 nm for the four rHDL species described in Table I; the measurements are not significantly different by unpaired t test analysis. The PAGE and gel filtration column measurements of the hydrodynamic diameters indicated that apoA-I (Δ44–126) formed a slightly larger complex than rHDL prepared with plasma apoA-I, while apoA-I (Δ139–170) and apoA-I (Δ190–243) both resulted in slightly smaller complexes. Again, the smaller hydrodynamic diameters of the latter two complexes may be attributed to some protein removal during migration through the gel matrix. Protein cross-linking was used to estimate the number of protein molecules per complex (Fig. 3). The rHDL comprised of intact apoA-I contained a mixture of particles containing 2 (70%) and 3 (30%) molecules of apoA-I per complex. The scans of the gels in Fig. 3 for the complexes containing the mutant proteins indicate that the cross-linking

table I

| Protein component of particle | Molar composition (POPC:FC:apoA-I) | Diameter of particle | No. of protein per particle | α-helix content |
|------------------------------|-----------------------------------|----------------------|-----------------------------|----------------|
| Plasma ApoA-I                | 100:5:1                           | 10.0 ± 0.3           | 2                           | 80 ± 6         |
| ApoA-I (Δ44–126)             | 100:5:1                           | 10.9 ± 0.7           | 4                           | 59 ± 16        |
| ApoA-I (Δ139–170)            | 100:5:1                           | 9.1 ± 0.1            | 3                           | 68 ± 0         |
| ApoA-I (Δ190–243)            | 100:5:1                           | 7.9 ± 0.2            | 3                           | 63 ± 3         |

*a* Determined from analysis on three separate reconstitution experiments (±1 S.D.) (n = 1 for apoAI (Δ190–243)). Final values were obtained after purification by gel filtration chromatography on a Superdex 200 column.

*b* Determined from nondenaturing polyacrylamide gradient gel electrophoresis using reference globular proteins.

*c* Average hydrodynamic diameter as measured on a Superdex 200 (Pharmacia) gel filtration column calibrated with reference globular proteins (±1 S.D.).

*d* Average major diameter of 100 particles determined from negative staining microscopy (±1 S.D.). Variances are not significantly different as determined by unpaired t test.

*e* Determined from SDS-polyacrylamide gel electrophoresis of delipidated apoAI or deletion variant after cross-linking with dimethyl suberimidate.

*f* Determined from molar ellipticities at 222 nm (±1 S.D.).

![Fig. 1. Elution profiles of POPC:FC:apoA-I reconstituted particles subjected to gel filtration chromatography. A 1 × 30-cm Superdex HR 200 (Pharmacia Biotech Inc.) gel filtration column was used to analyze solutions of reconstituted particles that were combined at a 100:5:1 PL:unesterified cholesterol:protein molar ratio. Fractions of 0.25 ml were collected and the protein was detected by absorbance at 280 nm. The void volume of the column was 8.1 ml, and the total volume was 20.1 ml.](http://www.jbc.org/content/282/25/23794/F1)
was incomplete. In these cases, the dominant cross-linked oligomer was assumed to represent the number of protein molecules on the discoidal particle (Table I). The predominant species were four molecules of apoA-I (D44–126), three molecules of apoA-I (D139–170), and three molecules of apoA-I (D190–243).

Circular dichroism was used to determine the effect of the various deletions in the apoA-I molecule on the average a-helix content of the proteins in the rHDL particles. Table I demonstrates that the a-helix content of the intact protein was greater than that of any of the apoA-I deletion mutants. The number of amphipathic helices per protein molecule can be predicted from the number of helical residues in the protein by assuming that there are approximately 22 amino acid residues per helical segment (28). On this basis, there are nine helices in the plasma apoA-I molecules in the rHDL particles; this number is in good agreement with prior work which suggest that with 75% a-helix there should be eight helical segments per protein molecule (22, 28). The equivalent numbers of helices for apoA-I (D44–126), apoA-I (D139–170), and apoA-I (D190–243) are 4, 7, and 5, respectively.

**Efflux of Cellular Cholesterol to Discoidal rHDL Particles**—To compare plasma apoA-I/FC/POPC and mutant apoA-I/FC/POPC discoidal complexes as acceptors of cell cholesterol, the rHDL were incubated with radiolabeled mouse L-cell fibroblasts at a concentration of 50 µg of PL/ml for periods of up to 6 h. Measurement of the fraction of radiolabeled FC present in the cells during the time course of incubation with rHDL particles showed that they exhibited similar abilities to remove cellular cholesterol (Fig. 4). Time courses of cholesterol efflux in the systems containing native apoA-I z FC z POPC and apoA-I (D190–243) z FC z POPC discoidal complexes are the same. Thus, the deletion of the carboxyl terminus did not impair the ability of the particle to participate in cholesterol efflux. Efflux to apoA-I (D44–126) z FC z POPC and apoA-I (D139–170) z FC z POPC rHDL was identical to efflux to particles containing intact apoA-I for the first 2 h of the experiment, after which point they showed some deviation from the control time course. After 6 h of incubation, apoA-I (D139–170) z FC z POPC rHDL removed slightly less FC and apoA-I (D44–126) z FC z POPC slightly more than the apoA-I z FC z POPC particles. All the time courses were fitted to a single exponential decay equation as described under “Methods” to obtain t1/2 values of efflux for each complex (Table II). An average t1/2 of 16.2 ± 1.8 h was measured, with maximum deviations of only 14% from this value. Comparison of the t1/2 of efflux by unpaired t test analysis indicates that none of the time courses involving incubation with apoA-I mutant-FC/POPC complexes were statistically different. These data indicate that the apoA-I deletions introduced here do not impair the functionality of the rHDL complexes as acceptors of cellular cholesterol.

**DISCUSSION**

**Formation of rHDL Particles**—The experiments summarized in Table I and Figs. 1–3 indicate that the deletion mutants...
medium. Test media consisted of rHDL at 50 particles described in this study, a "hinge-domain" extend stabilizing the protein in this close-packed conformation the perimeter of the complex. In this arrangement, electrostatic helices are actually present at the disc edge, it follows that the shown in Table III, with native apoA-I, 20 helices could theo- packed on a disc in such a manner that an equivalent number modification of the apoA-I molecule, the mutant proteins ical segments are present (Table III). Despite major structural present (see "Methods").

Since the deletion mutant proteins are not as tightly packed on the disc edge as intact apoA-I, because only 60–75% of the maximum possible number of helices were incorporated at the disc edge compared with the equivalent value of 90% for the intact apoA-I molecule. It seems that when helical segments are deleted in apoA-I, the interhelix attraction is reduced, resulting in a looser packing in the discoidal particles. In summary, all four types of apoA-I molecule formed similar particles suggesting that no specific amino acid sequence or helix-helix interactions are required for this process. The particles are stable provided that sufficient amphipathic 5-helices are present to cover the PL acyl chains at the disc edge.

Cholesterol Efflux to rHDL Particles—It is now generally accepted that efflux of plasma membrane cholesterol involves a passive desorption of FC molecules from the membrane fol- lowed by diffusion of these molecules through the aqueous phase surrounding the cells and incorporation of the FC into a PL-containing acceptor particle (1, 31). Studies have suggested that when FC acceptor particles contain apolipoprotein, there may be an interaction of the protein helices with specific lipid domains in the plasma membrane resulting in the modulation of cholesterol efflux (31–34), but the details of this association are not yet clear.

This study investigated the possibility that a particular region of apolipoprotein A-I is essential for the efficient release of cholesterol from the plasma membrane. Cholesterol efflux was measured to rHDL containing either wild-type apoA-I or one of the three apoA-I deletion mutants in a system in which other factors that could affect the efflux capability of the particles were eliminated. For example, acceptor particle PL properties and particle size have been shown to be determinants in cho- lesterol efflux (35, 36). In this study, the PL species was constant, and the acceptor particles were incubated with the cell monolayers at equal PL concentrations. In addition, differences due to varying sizes of the acceptor particles were not a factor here as the particles were constructed and isolated in a manner such that they were similar in size. Our study clearly demon- strates the need to isolate the rHDL particles from any free lipid or protein, as the gel filtration profiles indicated hetero- geneous preparations. The utilization of homogeneous accep- tors ensured that our results were not affected by the presence of these unreacted species. Having taken these factors into account, variations in the ability of the discs to remove chol- esterol could be attributed directly to the differences in protein structure.

The results indicate that the deletion of specific segments of apoA-I did not affect the ability of the protein to participate in cholesterol efflux when combined with lipid as a rHDL particle. The \( t_{1/2} \) values of efflux measured by Student's 

![Image](http://www.jbc.org/)

**Fig. 4.** Time course of free cholesterol efflux from mouse L-cell fibroblast to reconstituted HDL particles. Mouse L-cell fibroblasts grown to confluence in 22-mm tissue culture wells and trace-labeled with \(^{3}H\)-free cholesterol were incubated for 6 h at 37 °C with 1 ml of test medium. Test media consisted of rHDL at 50 µg PL/ml minimal essential medium, 0.5% bovine serum albumin. Symbols indicate the protein component of the particles: ▲, plasma apoA-I; ●, apoA-I (Δ44–126); ▼, apoA-I (Δ139–170); and ◇, apoA-I (Δ190–243). Each point represents the mean fraction of radiolabeled cholesterol detected in the media of three cell wells, and the error bars represent 1 S.D. The curves through the time points were obtained by fitting to a single exponential decay equation (see "Methods").
clonal antibodies were used to determine the regions of functional importance. Each of these studies identified a discrete region of apoA-I that was critical for apoA-I-mediated cholesterol efflux. It has been suggested that the region surrounding amino acid residue 165 of apoA-I (6) or the specific amino acid content in this position (7) determines the efficiency of apoA-I in cholesterol efflux. However, this region was deleted in this study in mutant apoA-I (Δ139–170) without any effect on the participation of the protein in the efflux process. Other regions of apoA-I have been suggested to be essential: amino acids 74–110 in studies involving rHDL species (5), and 137–144 in participation of the protein in the efflux process. Other regions of apoA-I have been suggested to be essential: amino acids 74–110 in studies involving rHDL species (5), and 137–144 in the HDL subspecies, pre-β HDL (8). These regions were addressed in this study with mutants apoA-I (Δ44–126) and apoA-I (Δ139–170), respectively, both of which when combined with lipid did not vary from the ability of intact apoA-I to remove cellular FC. Although small regions of the protein (1–44, 127–138, and 171–189) were not deleted in the mutants studied here, it seems likely that no specific domains of apoA-I are required for efficient apolipoprotein-mediated cholesterol efflux. The reasons for the variation in results between the present study and the monoclonal antibody studies are not entirely clear at this time. However, it is possible that inhibiting antibodies may be positioned in some cases such that they cover a significant portion of the polar phosphatidylcholine head groups, or the “faces,” of the discoidal complex. Since the deletion mutant proteins utilized here demonstrate looser helix packing at the disc edge than intact apoA-I, but the discs are equally good FC acceptors, cholesterol molecules probably do not incorporate at the disc edge, but rather by way of the face of the complex. If a monoclonal antibody covers a portion of the face, it follows that cholesterol incorporation will be inhibited.

The results of this study suggest that several structural features of the apoA-I molecule are not determinants of efficient cholesterol efflux. Thus, specific domains of the protein are not essential and, furthermore, interactions between specific helices seem not to be required. The number of protein molecules or helical segments per rHDL complex has no effect on the function of the particle in a cholesterol efflux system. It seems that the essential factor provided by apoA-I is the amphipathic helices required to stabilize the small rHDL particles. This agrees with previous work with synthetic peptides (4), which suggested that amphipathic helix interactions are essential for efflux, but that the number and amino acid sequence of the helices does not affect the process.

**Implications for Reverse Cholesterol Transport**—In addition to the phenomenon of peripheral cell FC transfer from the plasma membrane to HDL particles that has been described in this study, reverse cholesterol transport in vivo involves the subsequent steps of cholesterol esterification by LCAT and cholesterol ester transfer between lipoprotein particles via cholesterol ester transfer protein (33, 37, 38). Studies have indicated that discoidal HDL particles are better substrates for LCAT than spherical HDL particles (39). Although the abilities of the discoidal HDL particles containing either wild-type apoA-I or apoA-I deletion mutants to accept cholesterol molecules diffusing from the plasma membrane are similar, experiments of Holvoet et al. (10) indicate that discoidal particles containing these deletion mutants have different reactivities with LCAT. Thus, the conformation of apoA-I probably affects the second step of the reverse cholesterol transport process. The esterification by LCAT is essential to maintain the gradient of cholesterol flux from the cellular membrane (38, 40). These results suggest that the structure of apoA-I is critical for proper activation of LCAT but not for formation of HDL particles that can accept FC molecules diffusing from the plasma membrane of a cell.

The current results relate to cholesterol efflux from mouse L-cell fibroblasts where at least 60% of the plasma membrane cholesterol effluxes from a single kinetic pool under the experimental conditions utilized. However, some cell types appear to have several pools of plasma membrane cholesterol which may be accessed preferentially by apoA-I present in a particular conformation (32). Furthermore, it has been proposed that the conformation of apoA-I in pre-β HDL species is different to that in spherical HDL (33), and these small discoidal acceptors may access cholesterol in certain human fibroblast plasma membrane domains, such as caveolae, more efficiently (41). Further work is necessary to determine whether particular domains of the apoA-I molecule are important for efflux from these specific plasma membrane pools of cholesterol.

*Acknowledgments*—We thank Dr. Paul Holvoet of the Center for Molecular and Vascular Biology, University of Leuven, Belgium for his generous gift of the apoA-I deletion mutant proteins. We also thank Faye Baldwin, Sheila Benowitz, and Margaret Nickel for expert technical assistance.

**REFERENCES**

1. Johnson, W. J., Mahlberg, F. H., Rothblat, G. H., and Phillips, M. C. (1991) *Biochim. Biophys. Acta* 1085, 273–298
2. Sharrett, A. R., Patsch, W., Sorlie, P. D., Heiss, G., Bond, M. G., and Davis, C. E. (1994) *Arterioscler. Thromb.* 14, 1198–1104
3. Breslow, J. L. (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90, 8314–8318
4. Davidson, W. S., Lund-Katz, S., Johnson, W. J., Anantharamaiah, G. M., Palghatnarah, M. N., Segrest, J. P., Rothblat, G. H., and Phillips, M. C. (1994) *J. Biol. Chem.* 269, 22975–22982
5. Banks, C. L., Black, A. S., and Curtiss, L. K. (1994) *J. Biol. Chem.* 269, 10288–10297
6. Luchoomun, J., Theret, N., Clavey, V., Duchateau, P., Rosseneu, M., Brouwers, E., Demarsin, E., Engelborghs, Y., Rosseneu, M., Collen, D., and Brasseur, R., Benepe, F., Fruchart, J. C., and Castro, G. R. (1994) *Biochim. Biophys. Acta* 1212, 319–326
7. von Eckardstein, A., Castro, G., Wybranaka, I., Theret, N., Duchateau, P., Duverger, N., Fruchart, J.-C., Alhoud, G., and Assmann, G. (1993) *J. Biol. Chem.* 268, 2616–2622
8. Fielding, P. E., Kawanr, M., Catapano, A. L., and Phillips, M. C. (1994) *Biochim. Biophys. Acta* 33, 6981–6985
9. Sviridov, D., Pyle, L., and Fudge, N. (1996) *Biochemistry* 35, 189–196
10. Holvoet, P., Zhao, Z., Vankoo, B., Rov, R., Dherdier, D., Dhoest, A., Terevinne, J., Brouwers, E., Demarsin, E., Engelborghs, Y., Rosseneu, M., Collen, D., and Brasseur, R. (1995) *Biochemistry* 34, 13334–13342
11. Scain, A. M., and Edelstein, C. (1987) *Anal. Biochem.* 144, 576–588
12. Weisweiler, P., Friedl, C., and Ungar, M. (1987) *Clin. Chem. Acta* 169, 5

---

* TABLE III  

| Protein component of particle | Helical residues | α-Helices/protein molecule | Expt. number α-helices/particle | Maximum number α-helices/circumference |
|-----------------------------|----------------|--------------------------|-------------------------------|---------------------------------------|
| Plasma apoA-I              | 194            | 9                        | 14                            | 20                                     |
| apoA-I (Δ44–126)           | 95             | 4                        | 16                            | 25                                     |
| apoA-I (Δ139–170)          | 144            | 7                        | 21                            | 28                                     |
| apoA-I (Δ190–243)          | 119            | 5                        | 15                            | 25                                     |

* Determined from the percent α-helix content (Table I) and the total amino acid residues of the protein.

* a Number of helices was calculated from the number of helical residues assuming that each helix is 22 amino acids in length.

* b Determined from the percent α-helix content (Table I).

* c Calculated by multiplying the number in b by the number of protein molecules per disc (Table I).
ApoA-I Modification and Efflux of Cellular Cholesterol

249–254
13. Sparks, D. L., Phillips, M. C., and Lund-Katz, S. (1992) J. Biol. Chem. 267, 25830–25838
14. Markwell, M. A., Haas, S. M., Bieber, L. L., and Tolbert, N. E. (1978) Anal. Biochem. 87, 206–210
15. Sokoloff, L., and Rothblat, G. H. (1974) Proc. Soc. Exp. Biol. Med. 146, 1166–1172
16. Bligh, E. G., and Dyer, W. J. (1959) Can. J. Biochem. Physiol. 37, 911–917
17. Klansek, J., Yancey, P., St. Clair, R. W., Fischer, R. T., Johnson, W. J., and Glick, J. M. (1995) J. Lipid Res. 36, 2261–2266
18. Swaney, J. B. (1986) Methods Enzymol. 128, 613–626
19. Forte, T. M., and Nordhausen, R. W. (1986) Methods Enzymol. 128, 442–457
20. Sparks, D. L., Davidson, W. S., Lund-Katz, S., and Phillips, M. C. (1993) J. Biol. Chem. 268, 23250–23257
21. Johnson, W. J., Bannberger, M. J., Latta, R. A., Rapp, P. E., Phillips, M. C., and Rothblat, G. H. (1986) J. Biol. Chem. 261, 5766–5776
22. Jonas, A., Kezdy, K. E., and Wald, J. H. (1989) J. Biol. Chem. 264, 4818–4824
23. Nichols, A. V., Gong, E. L., Blanche, P. J., and Forte, T. M. (1983) Biochim. Biophys. Acta 750, 353–364
24. Sparks, D. L., Davidson, W. S., Lund-Katz, S., and Phillips, M. C. (1993) J. Biol. Chem. 268, 23250–23257
25. Schmidt, H.-J., Remaley, A. T., Stonik, J. A., Ronan, R., Wellmann, A., Thomas, F., Zech, L. A., Brewer, H. B., Jr., and Hoeg, J. M. (1995) J. Biol. Chem. 270, 5469–5475
26. J. Y., and Jonas, A. (1995) J. Biol. Chem. 270, 11290–11297
27. Palgunachari, M. N., Mishra, V. K., Lund-Katz, S., Phillips, M. C., Adeeye, S., O., Alluri, S., Anantharamaiah, G. M., and Segrest, J. P. (1996) Arterioscler. Thromb. Vasc. Biol. 16, 328–338
28. Segrest, J. P., Jones, M. K., DeLoof, H., Brouillette, C. G., Venkatachalanpathi, Y. V., and Anantharamaiah, G. M. (1992) J. Lipid Res. 33, 141–166
29. Rosseneu, M., Vanlee, B., Lino, L., Corin, J., Van Biervliet, J. P., Rauscher, J. M., and Brasseur, R. (1992) J. Biol. Chem. 270, 17106–17113
High Density Lipoprotein Particles in Cellular Cholesterol Efflux

Apolipoprotein A-I Structural Modification and the Functionality of Reconstituted

Kristin L. Gillotte, W. Sean Davidson, Sissel Lund-Katz, George H. Rothblat and Michael C. Phillips

J. Biol. Chem. 1996, 271:23792-23798.
doi: 10.1074/jbc.271.39.23792

Access the most updated version of this article at http://www.jbc.org/content/271/39/23792

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 40 references, 21 of which can be accessed free at
http://www.jbc.org/content/271/39/23792.full.html#ref-list-1