The Effect of Apolipoprotein A-II on the Structure and Function of Apolipoprotein A-I in a Homogeneous Reconstituted High Density Lipoprotein Particle

(Received for publication, July 3, 1997, and in revised form, September 29, 1997)

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In this study we examined the effects of apoA-II on the structure and function of apoA-I in homogeneous reconstituted HDL (rHDL). First, we measured the binding of apoA-II to apoA-I-rHDL, containing dipalmitylophosphatidylcholine or palmitoyloleoylphosphatidylcholine, and the degree of apoA-I displacement at various ratios of apolipoproteins. Using fluorescence methods, we determined that apoA-II binding is rapid, irreversible, and associated with apoA-I displacement only when the molar ratio of apoA-II/apoA-I is greater than 1:2. Next, we used the stable apoA-II/apoA-I-rHDL complex at the apoA-II/apoA-I ratio of 1:2 to examine its physical properties, apoA-I structure, and reactivity with lecithin: cholesterol acyltransferase (LCAT). Using chemical cross-linking in conjunction with fluorescence and electrophoretic methods, we demonstrated that the conformation of apoA-I must be flexible to allow apoA-II binding to the apoA-I-rHDL particles and showed that the hybrid particles have an unchanged Stokes diameter. Fluorescence and circular dichroism measurements revealed little or no change in the secondary structure or in the N-terminal domain of apoA-I, but showed a marked destabilization of apoA-I to denaturation by guanidine hydrochloride. Limited trypsic digestion indicated that the central region of apoA-I becomes accessible to proteolysis in the hybrid particles. Together, these results suggest that amphipathic α-helices of apoA-II replace four central helices of one apoA-I molecule (residues 99–187) in the complex and in the process destabilize apoA-I. Thus, apoA-II binding at physiological ratios may not completely displace apoA-I from HDL, but may provide a reservoir of easily exchangeable apoA-I. Finally, we showed that the reaction of the hybrid HDL with LCAT was inhibited 2–5-fold, relative to apoA-I-rHDL, due to a corresponding increase in the apparent $K_m$ value. This suggests that LCAT binding to the hybrid particles is sterically hindered by the excess protein (portions of apoA-I and apoA-II not bound to lipid). Therefore, apoA-II can modulate the reaction of HDL with LCAT by decreasing LCAT binding to hybrid particles and making the enzyme available for reaction with other substrates.

The main protein components of high density lipoproteins (HDL) are apolipoprotein A-I (apoA-I) and apolipoprotein A-II (apoA-II), which together comprise 70 and 20%, respectively, of the protein mass of HDL. These two apolipoproteins define two main classes of mature HDL, one which contains both apoA-I and apoA-II, and the other which contains apoA-I but does not contain apoA-II. The structure and function of apoA-I have been studied extensively. There is general agreement that apoA-I determines the size and shape of HDL and removes lipids (cholesterol and phospholipids) from cell membranes to give rise to preβ-1 HDL particles, which then transfer cholesterol to larger preβ-2 HDL discs. ApoA-I is also the main activator of the lecithin:cholesterol acyltransferase (LCAT) reaction, and is very likely the ligand in receptor recognition during HDL uptake by liver and steroidogenic cells. The study of apoA-I structure has been facilitated by the availability of defined reconstituted HDL (rHDL) particles. In discoidal rHDL, two or more molecules of apoA-I each containing from 6–8 anti-parallel amphipathic α-helices form a shell of protein around a phospholipid bilayer disc. The apoA-I structure is highly plastic and adaptable to the content and composition of the lipids it solubilizes (2, 3). Most known properties of apoA-I, including those observed in studies of transgenic mice for human apoA-I or knock-out mice (4, 5), strongly suggest an anti-atherogenic role for apoA-I in vivo. In contrast to apoA-I, much less is known about the structure and function of apoA-II. High density lipoproteins containing only apoA-II are not detected in the circulation, even though rHDL with only apoA-II can be readily prepared (3, 6). In vitro studies have demonstrated that apoA-II has a higher affinity for lipids than does apoA-I (7), and easily displaces apoA-I from native HDL and from rHDL particles (8–10). As in apoA-I, amphipathic α-helices (four helices) mediate the binding of apoA-II dimers to phospholipids. However, the physiologic function of A-II, aside from lipid solubilization, has not been firmly established. Several recent reports have addressed the role of apoA-II in HDL metabolism. Mowri et al. (11) have suggested a role for apoA-II in native HDL in the stimulation of the hepatic lipase reaction. Forte et al. (12) showed that lipid...

1 The abbreviations used are: HDL, high density lipoproteins; apoA-I, apolipoprotein A-I; apoA-II, apolipoprotein A-II; LCAT, lecithin:cholesterol acyltransferase; rHDL, reconstituted HDL; apoA-I-rHDL, HDL containing only apoA-I; GSH, guanidine hydrochloride; DPPC, 1,2-dipalmitylophosphatidylcholine; POPC, 1,2-palmitoyloleoylphosphatidylcholine; DNS-Cl, 1-dimethylaminonaphthalene-5-sulfonyl chloride; PRODAN, 6-propionyl-2-dimethylaminonaphthalene; BS3, bis(sulfosuccinimidyldimaleate); apoA-I-DPPC-rHDL, rHDL containing apoA-I and DPPC; apoA-I-POPC-rHDL, rHDL containing apoA-I and POPC; apoA-II-DNS, 5-dimethylaminonaphthalene-1-sulfonyl-labeled apoA-II; apoA-II/apoA-II-DPPC-rHDL, hybrid rHDL containing apoA-II, apoA-I, and DPPC; TPC, tosylphenylalanin chloromethyl ketone; PAGE, polyacrylamide gel electrophoresis.
free apoA-II could recruit phospholipid and cholesterol from cell membranes to the same extent as lipid-free apoA-I, suggesting that nascent apoA-II particles could form at the site of apoA-II biosynthesis and secretion. However, these authors also reported that the complexes of apoA-II with cellular lipids did not undergo the usual morphological changes seen with nascent apoA-I-HDL in the presence of LCAT. The latter observation is not surprising because it is well known that apoA-II is a very poor activator of LCAT (about 100-fold) in comparison with apoA-I (13). Most recently, Pussinen et al. (14) reported that apoA-II in plasma HDL inhibited the particle transformations catalyzed by the phospholipid transfer protein. In addition to these observations, the complex physiologic effects detected in apoA-II transgenic and knock-out mice suggest both anti-atherogenic and proatherogenic properties for apoA-II (15, 16).

Aside from its intrinsic function(s), apoA-II may exert its effects on HDL metabolism by modifying apoA-I structure and function when associated with HDL containing apoA-I. Past studies of the effects of apoA-II on apoA-I in HDL have been conducted under conditions that produce mixtures of particles with various apolipoprotein stoichiometries (10). Therefore, in this study our objective was to prepare homogeneous rHDL containing both apolipoproteins and to examine the properties of such particles and the effects of apoA-II on the structure and function of apoA-I.

**EXPERIMENTAL PROCEDURES**

**Materials**—Human apoA-I, apoA-II, and LCAT were prepared from blood plasma purchased from the Champaign County Blood Bank, Regional Health Resource Center. The purifications of apoA-I and LCAT were performed as described previously (17–19). The purification procedure for apoA-II was a modification of the protocol of Blanche et al. (18). After delipidating and purifying apoA-I from the HDL fraction (d = 1.063–1.212 g/ml), apoA-II was subsequently delipidated from the remaining HDL components by incubation with 6 mM guanidine hydrochloride (GuHCl) at 37 °C for 3 h, followed by ultracentrifugation at a density of 1.225 g/ml in a 55.2 Ti Beckman rotor at 50,000 rpm, 10 °C, for 48 h in the presence of GuHCl to prevent apoA-II from re-binding to the HDL particle fraction containing lipid-free apoA-II. This procedure resulted in a yield of apoA-II greater than 50% (versus 5 mM ammonium bicarbonate and lyophilized. The final purification of apoA-I and apoA-II was carried out by gel filtration in 3 M GuHCl using two Pharmacia Biotech Inc. Sephacryl HR-200 columns in tandem (2 × 90 cm, each).

L-a-Dipalmitoylphosphatidylcholine (DPPC), L-a-palmitoyl-oleoyl-phosphatidylcholine (POPC), crystalline cholesterol, and solid cholate were purchased from Sigma 1-dimethylaminonaphthalene-5-sulfonyl chloride (DNS-Ci) and 6-propionyl-2-dimethylamino- naphthalene (PRODAN) were obtained from Molecular Probes, Inc., Eugene, OR. Radiolabeled [14C]cholesterol was obtained from NEN Life Science Products. Bis(sulfosuccinimidyl)suberate (BS3) and polyvinylidene fluoride membranes saturated with MeOH to allow transfer of protein from the unreacted DNS-Ci. The molar concentration of apoA-II-DNS was calculated from the extinction coefficients of 12,000 M⁻¹cm⁻¹ for apoA-II at 276 nm; and 4300 M⁻¹cm⁻¹ and 1850 M⁻¹cm⁻² for DNS, at 276 and 340 nm, respectively (22, 23). The DNS/apoA-II molar ratio (labeling efficiency) was on average 1.1:1 (n = 6 experiments). This extent of labeling did not appear to affect the ability of apoA-II to bind lipoprotein, based on the similarity in size and composition of RHDL prepared with labeled or unlabeled apoA-II, or change the stability of labeled or unlabeled apoA-II associated with HDL in the presence of GuHCl.

Fluorescence Experiments—All fluorescence measurements were carried out on an ISS PCI photon counting spectrofluorometer at the Laboratory for Fluorescence Dynamics, University of Illinois, Urbana, IL. Fluorescence polarization data reporting on the binding of apoA-II-DNS to A-I-rHDL were acquired for samples in standard buffer at 20 °C in a thermostatted cell compartment.

The fraction of apoA-II that remained bound to lipid after addition of apoA-II-DNS to apoA-I-rHDL was determined from the change in wavelength of maximum fluorescence (λmax) of uncorrected emission spectra of the apoA-II tryptophan (Trp) residues, acquired within 2 min after the addition of GuHCl. The final concentration of GuHCl was 1.5 M, sufficient to denature lipid-free, but not lipid-bound, apoA-I. The emission spectra were obtained at room temperature using an excitation wavelength of 295 nm and an emission scan from 310 to 370 nm, with 1 nm excitation and emission slits. Under these conditions, Trp fluorescence is not detected, and only apoA-I Trp fluorescence is observed. The fraction of lipid-bound apoA-I (FLB) was calculated from the relationship:

\[
\lambda_{\text{FLB}} = \lambda_{\text{FLB}} + \lambda_{\text{LB}} (1 - F_B) = \lambda 
\]

where \( \lambda_{\text{FLB}} \) is the wavelength for 100% lipid-bound apoA-I in A-I-rHDL, \( \lambda_{\text{LB}} \) is the wavelength for lipid-free apoA-I, and \( \lambda \) is the wavelength of the apoA-II-DNS/A-I-rHDL solution, in the presence of 1.5 M GuHCl.

To detect changes in the hydration of the apoA-I-rHDL phospholipid surface upon binding of apoA-II, PRODAN in ethanol was added to apoA-I-DPPC-rHDL to give a molar ratio of DPPC/PRODAN of 600:1. After equilibration of the probe with the HDL, apoA-II was added and the emission spectra of PRODAN, excited at 390 nm, were recorded from 340–600 nm, at 5-min intervals.

**Circular Dichroism Measurements**—Circular dichroic (CD) spectra were recorded with a Jasco J720 spectropolarimeter at the Laboratory for Fluorescence Dynamics (University of Illinois). The samples were adjusted to a concentration of 0.100 mg/ml in 0.1 M phosphate buffer (pH 8.0). Measurements were made at room temperature using a 0.1-cm path length quartz cuvette. Spectra were acquired from 250 to 200 nm at a speed of 50 nm/min, with a 0.5-nm step resolution and a response time of 4.0 s, using a 1.0-nm bandwidth. Four scans were acquired and averaged for each sample; a base-line scan was subtracted to produce the final average scan. The molar ellipticity at 222 nm and the fraction of a-helical secondary structure were calculated as published previously (24).

**Native GGE and Western Blotting**—The procedure for cross-linking of apolipoproteins in rHDL with BS3 was published previously (24). Native gel of apoA-I-DPPC-rHDL, +BS3, and +apoA-II, was performed using non-denaturing 8–25% gradient gels on the Pharmacia Phast System. The gels were blotted for 10 min with polyvinylidene difluoride membranes saturated with MeOH to allow transfer of protein by diffusion. The membranes were subsequently reacted with antibod-
ies to apoA-I (1:5000 dilution) or apoA-II (1:1000 dilution) following the protocol for the ImmunoPure ABC peroxidase staining kit from Pierce. Horseradish peroxidase-conjugated secondary antibodies of goat anti-rabbit and rabbit anti-goat immunoglobulins were used to visualize the apoA-I and apoA-II primary antibodies, respectively. Diaminobenzidine tetrahydrochloride in 0.08% H₂O₂ was added as the horseradish peroxidase substrate. The Phast gels were stained with 0.1% Coomassie Blue R-350 after membrane blotting.

Limited proteolytic digestion of apoA-I-DPPC-rHDL was carried out in the absence and in the presence of apoA-II, to examine the susceptibility of apoA-I to proteolysis by trypsin. AI-DPPC-rHDL was incubated with apoA-II in a 2:1 molar ratio, apoA-I:apoA-II, for 20 min at 25 °C, then TPKC-trypsin was added to the sample in a ratio of 200:1, apoA-I:trypsin (w/w). Aliquots were withdrawn from the reaction mixture and from a control reaction mixture containing no apoA-II at time points of 5, 10, 30, and 120 min after the addition of trypsin. The digestion products were analyzed by SDS-PAGE using 20% homogeneous gels and the Pharmacia Phast System, followed by staining with 0.1% Coomassie Blue R-350 and/or Western blotting with antibodies to apoA-I or apoA-II. The gels were scanned with a Pharmacia LKB Ultrascan XL laser densitometer, and the sizes of the proteolytic fragments were determined by electrophoretic migration relative to low molecular weight standards (Pharmacia). The peaks for apoA-I identified at each time point on the scans represented intact apoA-I and apoA-I fragments of 22 kDa and of approximately 14 kDa. The 14-kDa fragment was subjected to N-terminal sequencing as follows: the 120-min digestion products were separated by SDS-PAGE using a discontinuous Tris-glycine system with a 12.5% T, 2.5% C resolving gel, and a 4% T, 2.5% C stacking gel. The bands were electrochemically transferred to a polyvinylidene difluoride membrane and stained; the 14-kDa band was excised and subjected to automated Edman degradation analysis on an Applied Biosystems 477A protein sequencer using eleven reaction cycles (Genetic Engineering Facility, University of Illinois Biotechnology Center).

LCAT Reaction Kinetics—Radiolabeled apoA-I-POP-C-rHDL or apoA-I-DPPC-rHDL, containing 5 mol of cholesterol/mol of apoA-I and [¹⁴C]cholesterol, were incubated with apoA-II for 20 min at 37 °C and subsequently reacted with LCAT as described previously (19). The lipids extracted in the organic phase were spotted on ITLC plates developed in petroleum ether/ethyl ether/acetic acid, 85:15:1, v/v. The lipid spots were cut out and the percent conversion of cholesterol ester from free cholesterol was determined by scintillation counting. Data analysis was performed using the KaleidaGraph 3.0.5, Abelbeck Software, Reading, PA.

RESULTS

Kinetics of ApoA-II Binding to ApoA-I-rHDL—We examined first the time course for the binding of apoA-II-DNS to apoA-I-DPPC-rHDL particles at several ratios of apoA-II/apoA-I. The results for a molar ratio of 1:2, apoA-II/apoA-I, are shown in Fig. 1. The fluorescence polarization of free apoA-II-DNS (0.180) increases rapidly to 0.240 and then more gradually over 30 min to an equilibrium value of 0.275, which is close to the fluorescence polarization of a control rHDL particle prepared with apoA-II-DNS (Fig. 1A). The increase in fluorescence polarization of apoA-II-DNS, upon binding to the rHDL, reflects the reduced rotational motions of the fluorophore in the large rHDL. Qualitatively similar kinetics were also observed for the binding of apoA-II-DNS to apoA-I-DPPC-rHDL or to apoA-I-POP-C-rHDL, at molar ratios from 0.25:1 to 2:1, apoA-II/apoA-I (data not shown). The binding of apoA-II-DNS to apoA-I-rHDL appears irreversible as dilutions of 20-fold over 4 h do not result in significant decreases in polarization from a value of 0.275.

B in Fig. 1 shows that when apoA-I is chemically cross-linked in the rHDL particles, the binding of apoA-II-DNS is markedly reduced. This indicates that the binding requires a structural rearrangement of apoA-I and, likely, does not occur on the exposed lipid surface of the particles.

C in Fig. 1 illustrates the effect of apoA-II binding on the lipid surface properties of the apoA-I-DPPC-rHDL. There is a rapid small (5-5 nm) blue shift in the fluorescence emission of the PRODAN probe upon apoA-II binding. This indicates a decrease in the hydration of the lipid and/or a change to a more condensed lipid state. However, the wavelength change is relatively small, suggesting that the lipid is not strongly perturbed by apoA-II binding. The two stages in the spectral changes coincide with the rapid and slower steps in apoA-II-DNS binding kinetics observed in A. These two kinetic steps may correspond to 1) a rapid initial binding to the edge of the rHDL, where apoA-I structural fluctuations permit apoA-II insertion and 2) a slower step requiring further structural rearrangements in apoA-I and/or apoA-II.

Ratio of ApoA-II/ApoA-I and ApoA-I Displacement—Fig. 2 shows the percent of apoA-I displaced from apoA-I-DPPC-rHDL when incubated with increasing amounts of apoA-II. Up to a molar ratio of 0.5:1 (or 1:2 in terms of rHDL stoichiometry) apoA-II/apoA-I, little or no apoA-I is displaced. At a molar ratio of 1:1, 50% of apoA-I is displaced; and at molar ratios greater than 2:1 essentially 100% of apoA-I is displaced. This corresponds to a stoichiometric displacement of one apoA-I molecule by two apoA-II molecules, as reported previously by other groups (8–10). The fact that there is minimal displacement of apoA-I at the 1:2 ratio of apoA-II/apoA-I indicates that apoA-II is distributed randomly among the apoA-I-rHDL particles. If
there had been a significant population of rHDL particles that bound two apoA-II molecules in this sample, it would have produced a corresponding amount of free apoA-I.

**Structural Properties of the Hybrid ApoA-II/ApoA-I-DPPC-rHDL**—To assess the diameter of the complex formed between apoA-II and apoA-I-DPPC-rHDL at the molar ratio of 0.5:1 (apoA-II/apoA-I), we performed nondenaturing gradient gel electrophoresis and detected the protein by Coomassie stain and by Western blotting (Fig. 3). The hybrid particle was compared with the control apoA-I-DPPC-rHDL and with samples of both particles that were chemically cross-linked. The hybrid particle (lane 5) has the same Stokes diameter as the control rHDL (lane 2); the hybrid contains all of the added apoA-II, but loses significant amounts of apoA-I upon electrophoresis. Apparently, the amount of free apoA-I in the hybrid sample is significantly larger than that detected in Fig. 2 and indicates that destabilized apoA-I is stripped off during electrophoresis. All the cross-linked rHDL particles appear to have Stokes diameters several angstroms smaller than the rHDL that have not been cross-linked, probably because chemical cross-linking produces more compact particles that migrate further in the gel. The cross-linked hybrid particle does not lose apoA-I during electrophoresis (lane 3), whereas a cross-linked apoA-I-rHDL particle to which apoA-II was added later (lane 4) incorporates much less apoA-II. This observation confirms the result presented in Fig. 1B, that previous immobilization of apoA-I in the rHDL prevents binding of apoA-II.

Table I summarizes several physical properties of the hybrid apoA-II/apoA-I-DPPC-rHDL in comparison with the apoA-I-DPPC-rHDL control. The hybrid particle has the same major dimension (i.e. disc diameter) as apoA-I-DPPC-rHDL and is homogeneous, since the free apoA-I seen in lane 5 of Fig. 3 is an artifact of electrophoresis. The stoichiometry of two molecules of apoA-I and one molecule of apoA-II was confirmed by performing SDS-PAGE on the cross-linked hybrid, showing that the major product band had a molecular weight of 73 kDa (data not shown).

The fluorescence spectra of the Trp residues in the hybrid particle were essentially identical to apoA-I-rHDL, suggesting that the three-dimensional environment of the Trp residues in the apoA-I N-terminal region had not been affected by the incorporation of apoA-II. The average α-helix content of the apolipoproteins in the hybrid corresponds to the value expected (62%) if both apoA-I and apoA-II had the highest α-helix content measured in their respective lipid-bound states (69 and 49%). Apparently, the incorporation of apoA-II into the complex does not change the total α-helix content of apoA-I. However, the presence of apoA-II in the hybrid particles alters drastically the denaturation behavior of apoA-I by GuHCl. While apoA-I in the control particles has a midpoint for denaturation at 6.1 M GuHCl, the apoA-I in the hybrid particles has a midpoint for denaturation at 3.4 M GuHCl. Clearly apoA-II destabilizes apoA-I in the hybrid particles as already suggested by the stripping of apoA-I during electrophoresis.

**Limited Proteolytic Digestion**—To confirm the effect of apoA-II on the stability of apoA-I in hybrid particles and to obtain some information on the region(s) of apoA-I that may be more exposed to proteolysis after apoA-II binding, we performed limited digestion of the rHDL with trypsin, as reported previously by our laboratory (25). The time course of tryptic digestion was followed over 2 h, and the products of the reaction were analyzed by SDS-PAGE (Fig. 4). The results show that in the control particles apoA-I is considerably more resistant to trypsin than in the hybrid particles. While about 25% of apoA-I is still intact in apoA-I-DPPC-rHDL after 2 h, most of the apoA-I has been digested in the hybrid particles. Formation of the 22-kDa fragment of apoA-I followed the same kinetics for both particles (data not shown), suggesting that the structure and exposure to the proteolytic residues in apoA-I (25) is the same. Furthermore, about 40% of the digested apoA-I from the apoA-II/apoA-I-DPPC-rHDL particles appears as a 14-kDa fragment approximately half the size of the 28-kDa intact apoA-I. This fragment is hardly detectable in the apoA-I-DPPC-rHDL digest without apoA-II. Edman degradation of this fragment showed that it contains the N terminus of intact apoA-I. Apparently a new accessible tryptic site is exposed.

**TABLE I**

| Property                        | ApoA-II/ApoA-I-DPPC-rHDL | ApoA-I-DPPC-rHDL |
|---------------------------------|--------------------------|-----------------|
| Stokes diameter (Å)             | 98 ± 2                   | 98 ± 2          |
| Composition (molecules/rHDL)    | 2                         | 1               |
| ApoA-I                          | 1                         | 1               |
| Trp wavelength max. fl. (nm)    | 330 ± 1                  | 331 ± 1        |
| α-Helix content (%)             | 63 ± 2                   | 69 ± 3         |
| [GuHCl] at 50% denaturation (M) | 3.4                      | 6.1            |

- From migration on nondenaturing GGE (see Fig. 3). Mean (±S.D.).
- From the stoichiometry of the preparation, also from cross-linking with BS3 and SDS-PAGE (24).
- From uncorrected fluorescence spectra excited at 295 nm. The uncertainty of the measurements of the wavelength of maximum fluorescence is ±1 nm.
- From CD measurements and the Chen et al. (41) empirical expression for fraction of α-helix. Mean (±S.D.).
- From uncorrected fluorescence spectra excited at 295 nm and measured at guanidine hydrochloride concentrations ([GuHCl]) from 0.72 to 6.37 M.
around the middle of the molecule in the presence of apoA-II. The amino acid sequence of apoA-I contains tryptic sites in this region, at Arg-116, Lys-118, and Arg-123. These results confirm the destabilizing effect of apoA-II on apoA-I in the hybrid particles and suggest that the N- and C-terminal regions of apoA-I are similarly protected or exposed to trypsin, while the central region of apoA-I becomes much more susceptible to proteolysis in the hybrid particles.

Reaction Kinetics with LCAT—Finally, we measured the reaction kinetics of LCAT with hybrid apoA-II/apoA-I-DPPC-rHDL and with apoA-II/apoA-I-POPC-rHDL particles in comparison with the corresponding control apoA-I-rHDL particles. Even though most of the experiments described above were performed with particles prepared with DPPC, it is well known from our past work that these are less than optimal substrates for LCAT (26). Therefore, we also included POPC-cholesterol-rHDL as substrates in this experiment. We had shown previously that these particles bind apoA-II just as well as apoA-I-DPPC-rHDL.

Fig. 5 shows the inhibitory effect of apoA-II on the reaction of apoA-I-POPC-rHDL with LCAT. The results with the apoA-I-DPPC-rHDL were quite similar (not shown). Table II summarizes the apparent kinetic constants for these reactions. The apparent $V_{\text{max}}$ values, which reflect the LCAT activation step and the catalytic step in the Verger and de Haas kinetic model (27, 28), are almost the same for the hybrid and the control particles. In contrast, the apparent $K_m$ values, which reflect the enzyme binding step and the catalytic step, are increased 2- and 5-fold for the hybrid particles. The discrepancy in magnitude for the DPPC, compared with the POPC-containing particles, is probably due to the lower efficiency of the DPPC-rHDL as substrates for the LCAT reaction. Since the catalytic steps are likely to be identical for the hybrid and control particles, it appears that the binding of LCAT to the hybrid particles is affected by the presence of apoA-II. Possibly the central portions of apoA-I displaced from contact with lipid by apoA-II, and the N- and C-terminal regions of apoA-II that do not bind to lipid (29, 30) interfere sterically with LCAT binding to the hybrid particles. However, the LCAT that binds could be normally activated by the one molecule of apoA-I that is not affected by apoA-II binding.

DISCUSSION

The main conclusions from this work are the following: 1) binding of apoA-II to apoA-I-rHDL is rapid, followed by a slower structural rearrangement that reaches a stable state in about 30 min. The binding of apoA-II appears irreversible. 2) At molar ratios of apoA-II/apoA-I, 0.5:1 or lower, binding of apoA-II occurs without displacement of apoA-I; at higher molar ratios, apoA-II displaces apoA-I from apoA-I-rHDL particles in a stoichiometry of two apoA-II per one apoA-I. 3) Binding of apoA-II requires conformational mobility of apoA-I and leads to its destabilization on the apoA-I-rHDL particles. 4) The region of apoA-I affected by the binding of one molecule of apoA-II is its central region, probably involving the mobile hinge domain (31). Neither the N-terminal nor the C-terminal regions of apoA-I appear to be affected by apoA-II binding. 5) Binding of one molecule of apoA-II per apoA-I-rHDL inhibits the reaction of the rHDL with LCAT by increasing the apparent $K_m$. This suggests a decreased affinity of the enzyme for the particles. An unchanged apparent $V_{\text{max}}$ on the other hand, indicates that apoA-II binding does not affect enzyme activation nor the catalytic steps of the reaction.

Because this study was performed with discoidal rHDL particles, these findings are especially relevant to native nascent HDL, but they also should apply to mature HDL. In vivo the molar ratio of apoA-II/apoA-I in humans is around 0.5:1 (32); therefore, the conditions prevail for binding of newly synthesized apoA-II to preexisting apoA-I-rHDL leading to apoA-I destabilization rather than to complete displacement. The destabilized apoA-I probably represents part of the “weakly
bound" apoA-I that is readily stripped from HDL during preparative (e.g. ultracentrifugal) or analytical (e.g. electrophoretic) procedures (33). In fact, even immunoaffinity separations of rHDL subclasses can result in apoA-I stripping as we observed in work related to this project. We attempted to fractionate mixtures of apoA-II/apoA-I-rHDL and found that compositional changes occurred with time and number of passages through immunoaffinity columns, characterized by the loss of lipid-free apoA-I. Thus, in vivo hybrid HDL particles probably exist with one molecule of apoA-I when they are newly formed or with two molecules of apoA-II after one molecule of apoA-I has been fully displaced. In addition to destabilizing apoA-I, the binding of apoA-II to nascent apoA-I-HDL would have a significant impact on their reaction with LCAT, causing as much as a 5-fold decrease in reactivity. These effects of apoA-II could be both anti-atherogenic and proatherogenic: anti-atherogenic, because destabilization, and eventual displacement of apoA-I, would provide a pool of lipid-free apoA-I that could form new nascent HDL particles (12), and proatherogenic, because the LCAT reaction that is key in reverse cholesterol transport would be inhibited (34).

A model for the complex of one molecule of apoA-II with the apoA-I-DPPC-rHDL particles is represented in Fig. 6. This model particle has a similar Stokes diameter to the initial rHDL because the equivalent of four helical segments of apoA-II have replaced four helices of apoA-I on the periphery of the disc. The helices of apoA-I removed from contact with lipids probably include the putative mobile, hinged region of apoA-I (31, 35) (residues 99–143). This region of apoA-I is implicated because proteolytic cleavage is promoted in the center of apoA-I’s sequence in the apoA-II/apoA-I-rHDL, while the N- and C-terminal regions are not affected. Since there are no changes in the fluorescence spectra of the Trp residues of apoA-I when apoA-II binds, it seems likely that the other two helices of apoA-I that are displaced by apoA-II are those C-terminal to the hinged region rather than N-terminal. Although displaced from contact with lipid, residues 99–185 in the affected apoA-I molecule still retain α-helical structure. This sequence of apoA-I may adopt a helical bundle structure in solution; however, this may affect the hydrodynamic properties of the particles. Another possibility is that the free helices bind weakly to the lipid headgroup region of the discs, as we have demonstrated with excess free apoA-I added to apoA-I-rHDL. Finally, the lipid-free sequences of apoA-I may interact with the globular N-terminal domain of apoA-I or with lipid-free regions of the apoA-II molecule. In any case, the resulting structure has the same major dimension as the initial apoA-I-rHDL. A recent report on a new point mutant of apoA-I, A-I (R160L)Oslo, which is associated with low levels of hybrid apoA-II/apoA-I-HDL in plasma (36), lends support to the model depicted in Fig. 6. The change of an Arg to a Leu residue in position 160 would increase the hydrophobicity and possibly the stability of the interaction of the corresponding amphipathic helix with lipid. This increased stability of the central region of apoA-I could prevent the binding of apoA-II to the apoA-I-HDL and lead to decreased levels of hybrid particles.

In the model one apoA-I molecule is modified in the region that has been implicated in the activation of LCAT (37–39); however, the second apoA-I molecule is unaffected and could activate the reaction normally after LCAT binding to the rHDL. Nevertheless, reactivity with the enzyme would be decreased as a result of steric interference by lipid-free regions of apoA-I and apoA-II with the binding of LCAT to the lipid surface of the particle (40).

In this study we report for the first time on the effects of apoA-II on the structure and function of apoA-I in a homogeneous hybrid particle. We show that apoA-I adopts a distinct, destabilized conformation upon apoA-II binding to apoA-I-rHDL. This destabilized apoA-I is a potential source of easily exchangeable apoA-I. ApoA-II also modulates LCAT activity on hybrid HDL particles by decreasing its binding affinity.

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Effect of ApoA-II on ApoA-I Structure and Function

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