The Number of Amphipathic $\alpha$-Helical Segments of Apolipoproteins A-I, E, and A-IV Determines the Size and Functional Properties of Their Reconstituted Lipoprotein Particles*

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The objective of this work was to determine the role of the amphipathic $\alpha$-helical structural units of human apolipoproteins A-I, E, and A-IV in defining the sizes and reactivities with lecithin:cholesterol acyltransferase (LCAT) of their reconstituted lipoprotein particles. We prepared reconstituted high density lipoprotein (rHDL) particles with each of the three apolipoproteins in two weight ratios with lipid: 2.7/0.07/1 and 1.35/0.04/1, palmitoyloleylphosphatidylcholine/cholesteryl/apolipoprotein, by the sodium cholate dialysis procedure; and examined the rHDL product sizes and distributions by nondenaturing gradient gel electrophoresis. The rHDL particles were also incubated with low density lipoprotein (LDL), and with LDL plus LCAT, to observe any structural modifications due to phospholipid transfers to LDL and to cholesterol esterification by LCAT. In addition, we examined the average structural properties of the original rHDL by several fluorescence methods and circular dichroism spectroscopy, and determined their reaction kinetics with LCAT.

The results indicate that the diameters of the largest rHDL particles, containing two apolipoproteins per particle, correlate with the maximum number of putative amphipathic $\alpha$-helical segments in their sequences, and that smaller particles of this class may arise from the removal of one or more $\alpha$-helical segments from contact with lipid. Furthermore, the larger particles may be converted into the smaller ones upon loss of phospholipid to LDL, and may form one or two well defined products when reacted with LCAT. In general, the subclasses of particles have distinct spectroscopic properties, consistent with a different apolipoprotein folding in particles containing different proportions of phospholipid to apolipoprotein. Furthermore, the different apolipoprotein structures lead to significant differences in reactivity with LCAT.

Apolipoproteins are the functional components of lipoproteins. They determine the structure of the lipoprotein particles and their metabolism, starting from the synthesis and secretion of lipoproteins from specific cells, through the enzymatic reactions and lipid transfers that lipoproteins undergo in circulation, ending with the binding of lipoproteins to cellular receptors and their catabolism. The realization that the same apolipoprotein can exist in diverse functional states depending on the lipoprotein class or subclass on which it is found (1–3) has spurred interest in the detailed molecular properties of the apolipoproteins, and the relationships between their structure and function. However, because native lipoprotein classes are extremely heterogeneous in composition and in particle size distributions, the only practical way of obtaining such information is by using reconstituted, chemically and physically defined lipoproteins (4–6).

For apolipoprotein A-I (apoA-I),1 our laboratory (2, 7) and others (8–10) have shown that discoidal reconstituted HDL (rHDL) can be prepared with pure phosphatidylcholines (PC) in discrete particle sizes. We recently showed that rHDL classes with 2 and 3 molecules of apoA-I contain subclasses which can be isolated and which have distinct and reproducible structural and functional properties (2, 5, 6). On the basis of those observations, we proposed a model for the rHDL discs where the 22-amino acid amphipathic $\alpha$-helical segments of apoA-I (11, 12) are packed side-by-side in an antiparallel fashion and are oriented parallel to the acyl chains of the phospholipids around the periphery of the rHDL disc. Subsequently, similar models were proposed by Brasseur et al. (13) from molecular modeling and by Marcel et al. (14) from monoclonal antibody binding studies. Our model accounts for the different, discrete diameters of the rHDL particles by the number of $\alpha$-helical segments of apoA-I that are in contact with the lipid, and correlates with the ability of apoA-I to activate lecithin:cholesterol acyltransferase (LCAT) in the different structural states (2, 5, 6).

Apolipoproteins E and A-IV (apoE, apoA-IV), which arose from the same ancestral gene as apoA-I (12, 15, 16), also contain 22-amino acid repeated sequences which probably account for the lipid binding (17, 18) and LCAT activating (19, 20) properties of these apolipoproteins.

The objective of this study was to determine whether human apoE and apoA-IV, like apoA-I, form discrete rHDL particles with distinct structural and functional properties which are compatible with the model for apolipoprotein folding in the discoidal rHDL, proposed for apoA-I (2).

**EXPERIMENTAL PROCEDURES**

**Apolipoprotein and rHDL Preparations—**Human apoA-I was prepared from human plasma donated by the Champaign County Blood Bank by the routine method used in the Jonas laboratory based on the procedures of Edelstein et al. (21). Human apoA-IV and apoE

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1 The abbreviations used are: apoA-I, apolipoprotein A-I; HDL, high density lipoprotein; rHDL, reconstituted HDL; PC, phosphatidylcholine; POPC, palmitoyloleyl-PC; LCAT, lecithin:cholesterol acyltransferase; apoE, apolipoprotein E; apoA-IV, apolipoprotein A-IV; GdnHCl, guanidine hydrochloride; LDL, low density lipoprotein.

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apoE was separated from other very low density lipoproteins apolipoproteins by preparative SDS-polyacrylamide gel electrophoresis (22). ApoA-IV was derived from the phospholipid emulsion and was further purified by anion-exchange chromatography on a Mono-Q column using the Pharmacia FPLC system (23). The purity of the apolipoproteins was checked by SDS-PAGE and was found to be greater than 95%. Prior to use in the preparation of the rHDL particles, the biolayered proteins were solubilized in 4 M guanidine hydrochloride (GdnHCl) in 10 mM Tris-HCl, pH 8.0, buffer, containing 0.15 M NaCl, 0.01% EDTA, and 1 mM Na3VO4, and were then dialyzed against the same buffer without the detergent. This buffer was used in all the experiments except where a different buffer is specifically indicated. The concentration of the apolipoproteins was obtained from their absorbance at 280 nm, and the following extinction coefficients: apo-AI, 1.13 mg cm−1 (24); apoE, 1.32 mg cm−1 (24); apo-AIV, 0.38 mg cm−2 (based on the amino acid composition of apo-AIV and the absorbance properties of Tyr and Trp) (25).

The discoidal rHDL particles were prepared by the Na cholate dialysis method (4) using 1.3 mg of apolipoprotein in each preparation and two distinct weight ratios 50/2.5/1, POPC/cholesterol/apo, respectively. About 4,700 cpm of apoA-I these weight ratios correspond to molar ratios of 100/5/1, and comparable rHDL complexes with different apolipoproteins. For dialysis to remove the Na cholate, the clear rHDL preparations were used to solubilize the lipids during the preparation. After extensive denaturing gradient gel electrophoresis using PAA 4/30 gels from described in this study.

The rHDL particle sizes and distributions were analyzed by non-denaturing gradient gel electrophoresis using PAA 4/30 gels from Pharmacia LKB Biotechnology Inc., run in a Tris/HEPES buffer, at 150 V for 19 or 8.5 h. The gels were stained with Coomassie Blue and were scanned with an LKB Ultro Scan XL laser densitometer. Protein standards with the following Stokes diameters were included in each gel: bovine serum albumin (71 Å), lactate dehydrogenase (82 Å), casein (72 Å), horse ferritin (122 Å), and thyroglobulin (170 Å), supplied in the Pharmacia Fine Chemicals calibration kit. The standard curves generated from these globular proteins were used to estimate the diameters of the discoidal rHDL. The estimates should be adequate since the axial ratios of the discs do not exceed 3/2 and the gels are run to equilibrium.

In addition to the original rHDL samples, rHDL samples incubated with low density lipoprotein (LDL), and with LDL plus LCAT were examined by nondenaturing gradient gel electrophoresis. The human LDL prepared by sequential ultracentrifugal flotation was heat treated (50 °C, 1 h) and added to the rHDL preparations in a 5-fold excess, by weight, over the rHDL protein. Pure LCAT was added in 0.2-μg amounts per 10 to 15 μg of rHDL protein. Incubations were performed at 37 °C for 24 h.

To determine the number of apolipoprotein molecules per particle, the original rHDL preparations and free apolipoproteins were cross-linked with bis-(sulfosuccinimidy1)suberate according to the procedure of Staros (26). The products of the cross-linking reaction were analyzed by SDS PAGE on PAA 4/30 gradient gels.

Spectroscopic Measurements—Uncorrected fluorescence spectra were recorded with the Perkin-Elmer MPF 66 spectrophotometer at ambient temperature (24 °C). Exciting wavelengths of 280 and 295 nm, and emission slits were employed. The GdnHCl-containing solutions, solid GdnHCl in appropriate amounts was added sequentially to the fluorescence cuvette containing 0.70 ml of the rHDL solution adjusted to an absorbance of 0.10 at 280 nm. Mixing and recording of the spectra for each concentration of GdnHCl was performed. Since GdnHCl has denaturant properties that are time and temperature-dependent (5), we estimated that this sequential method of GdnHCl addition causes insignificant temperature effects and minimal time effects below 3 M GdnHCl concentrations. Above 4 M GdnHCl, the total time of exposure to the denaturant (about 20–30 min) results in a 10–20% overestimation of denaturation relative to that at time 0. This deviation is comparable for all the samples and is acceptable in view of the great savings of sample solutions.

Fluorescence polarization values were measured with the SLM Model 400 polarization instrument using a 280-nm excitation wavelength, 4-nm slits, and Corning C-52 emission filters, at a temperature of 24 °C. The same solutions were used in these measurements as in the recording of fluorescence spectra. Circular dichroism spectra were recorded with a Model CD6 Spex Industries (Edison, NJ) spectropolarimeter at the Laboratory for Fluorescence Dynamics, University of Illinois, Urbana, using a 1-mm quartz cuvette, and rHDL solutions with an absorbance at 280 nm of 0.05.

The % content of α-helical structure was estimated from the molar ellipticity values at 222 nm using the expression of Chen et al. (27). Denaturation with GdnHCl was performed by the same method as that used in the fluorescence experiments (i.e. solid GdnHCl was added sequentially to 0.70 ml of rHDL solution).

Reaction with LCAT—LCAT was purified as described previously (28) to a maximum specific activity of 34 amol of cholesterol ester/h/μg, which remained essentially constant for 6 months. During the preparation and the activity determination of LCAT, a standard rHDL preparation containing 100/10/1 molar ratio of egg PC/cholesteryl/apoA-I and radiola beled cholesterol (14C)cholesteryl was employed.

The measurements of the reaction kinetics of the rHDL particles with LCAT were performed under conditions where the reaction rates are linear (below 20% cholesterol ester formation) and proportional to the enzyme concentration. The apolipoprotein concentrations ranged from 1.8 × 10−11 to 2.6 × 10−10 M in the reaction mixtures, which also contained 4% bovine serum albumin, 1 mM β-mercaptoethanol, and 20 ng of LCAT in a total volume of 0.5 ml of the Tris buffer. The reactions were performed at 37 °C for 20 to 40 min, and were stopped with 5 ml of chloroform/methanol (2/1, v/v). The extraction, TLC, and scintillation counting of the lipids was performed as described previously (28). The analysis of the kinetic results was performed by the Lineweaver-Burk method using linear regression analysis of the data.

RESULTS

Particle Sizes and Their Distributions—The sizes and distributions of the rHDL particles were examined by nondenaturing gradient gel electrophoresis. Gels run for 8.5 h (not shown) indicated that only the rHDL-2 preparation with apo-A-I (1.35/1, PC/apoA-I, mg/mg) contained about 17% of free apolipoprotein, all the other preparations had incorporated the apolipoproteins completely into the complexes with lipids. Gels run to equilibrium for 19 h are shown in Fig. 1. Panel A shows the original rHDL samples: the average particle

![Fig. 1. Nondenaturing gradient gel electrophoresis of rHDL particles prepared with apoA-I, apoE, and apo-A-IV.](image-url)
sizes increase with increasing proportions of PC to apolipoprotein and with increasing molecular weight of the apolipoprotein. The pairs of rHDL preparations with the same apolipoprotein contain particles of equivalent sizes but markedly different distributions. Panel B shows the rHDL samples after incubation with LDL. Changes in particle distributions are evident compared to the original rHDL samples. From our previous work with rHDL complexes containing apoA-I (29, 30), we know that LDL is an excellent donor of unesterified cholesterol to and acceptor of phospholipid from rHDL. A net transfer of 30–50% of rHDL phospholipid to LDL leads to rHDL particle rearrangements and structural changes in apoA-I, which have been extensively described (29, 30). In this experiment, as in our previous work, the 94-Å particles containing apoA-I upon loss of PC to LDL, rearrange to 87 Å, and ultimately to 78- and 106-Å species. The apoE containing particles change in their distribution less than the apoA-I particles; nevertheless, the 87-Å particles become relatively more prominent compared to the original rHDL. The rHDL particles with apoA-IV, like those with apoA-I, form smaller and larger species upon incubation with LDL. The particles with diameters of 108 and 154 Å are probably analogous to the 78- and 106-Å particles containing apoA-I, respectively, in that they represent the most stable particle subtypes upon depletion of PC. In the rearrangement process, some apoA-IV is released. Panel C of Fig. 1 presents the results of the incubation of the rHDL particles with LDL and LCAT. The products of the enzymatic reaction give distinct size distribution patterns compared to the original rHDL or those incubated with LDL alone. For the apoA-I containing particles the main product is 92–94 Å in diameter, this 93-Å spherical product has been well characterized (6). The shoulder at 108 Å is probably an intermediate, and the 76-Å particle has not yet been analyzed by us but, very likely, it is analogous to the particle with two apoA-I molecules described by Ritter and Scanu (31). The apoE containing rHDLs give a single product with a diameter of 104 Å and the apoA-IV containing particles form two products, one 113 Å and the other 134 Å in diameter. Whether or not these particles represent the final spherical products of the LCAT reaction remains to be determined in future work, but considering the good reactivity of the original rHDL with LCAT, a 24-h incubation in the presence of LDL as the cholesterol donor should be sufficient to complete the reaction. The diameters of the major particles with all three apolipoproteins are summarized in Table I.

All the original rHDL particles cross-linked with bis-(sulfo succinimidyl)suberate indicate the presence of two apolipoprotein molecules per particle.

Spectral Properties—Although the rHDL preparations contain mixtures of particles which may have distinct structures and spectral properties, as is the case with rHDL containing apoA-I (29), the major particle species represents from 41 to 75% of the total apolipoprotein mass in each preparation (Table I). Therefore, the average spectral properties can reveal structural differences among rHDL populations and may be compared with the behavior of the free apolipoproteins in solution.

Table II summarizes the results of several fluorescence experiments and CD measurements. The wavelengths of maximum fluorescence reflect the polarity of the local environment of the Trp residues. In the rHDL containing apoA-I and apoE, the wavelengths are shifted a few nanometers to the blue, and the fluorescence intensities are increased about 10–30%, relative to the free apolipoproteins. The rHDLs prepared with apoA-IV, as well as free apoA-IV, have an unusual bimodal Trp fluorescence spectrum with maxima at 337 and 348 nm. The relative intensities of these two peaks change upon binding to lipid, but in the opposite direction to that expected; that is, in the free protein the 337-nm peak is more prominent. In addition, there is a major 110% increase in fluorescence intensity going from the free protein to the rHDL state. Apparently, the single Trp residue of apoA-IV (Trp2) is present in two distinct ensembles in the dimeric protein in solution (32), and in the two apolipoprotein molecules found in each rHDL particle. The unusual behavior upon binding to lipid reflects major structural changes in the apolipoprotein, but does not support the view that the Trp residues are involved in contacts with lipid. The fluorescence polarization results strengthen the observation that major structural differences exist between the free apolipoproteins and the lipid-bound forms, and that structural differences also exist between the different rHDL particles containing the same apolipoprotein.

The % α-helix estimates give the expected increases in α-helix content upon binding to lipid for the A-I and E apolipoproteins, but show a significant decrease for apoA-IV. This may be explained by the loss of the secondary structure of the apoA-IV dimer present in solution when the apolipoprotein binds to a micellar lipid surface (33). This interpretation is consistent with the change in relative fluorescence intensity at 337 versus 348 nm upon binding of apoA-IV to lipid.

Denaturation with Guanidine Hydrochloride—Fluorescence emission and CD spectral changes were used to monitor the denaturation of the free apolipoproteins and the rHDL particles by increasing concentrations of GdnHCl. It is evident from Figs. 2, 3, and 4 that the free proteins denature at much lower GdnHCl concentrations than the corresponding lipid complexes. The GdnHCl concentrations for 50% denaturation are listed in Table III. For all the rHDL particles, disruption of the secondary structure of the apolipoproteins is essentially complete by 5 mM GdnHCl, whereas the Trp fluorescence indicates that Trp residues remain partially protected from

| rHDL | Apo | PC/Apo | Original | Incubated with LDL | Incubated with LDL + LCAT |
|------|-----|--------|----------|-------------------|--------------------------|
|      |     | mg/mg  | rHDL     |                   |                          |
| 1    | A-I | 2.71/1 | 94 (49%) | 79, 108           | 94                       |
| 2    | A-I | 1.35/1 | 77 (54%) | 77                | 72, 76                   |
| 3    | E   | 2.71/1 | 109 (41%)| 108               | 105                      |
| 4    | E   | 1.35/1 | 87, 107 (42, 58%) | 87, 104            | 103                      |
| 5    | A-IV| 2.71/1 | 145 (49%)| 154               | 134                      |
| 6    | A-IV| 1.35/1 | 116 (75%)| 108               | 113                      |

*PC/apo weight ratios are those of the original reaction mixtures. For the apoA-I HDL-1 and rHDL-2 preparations these ratios are equivalent to 0:0/1 and 50:1 molar ratios, respectively.

The original rHDL preparations for each apolipoprotein consist of multiple particle populations, the diameter of the main one is given; in parentheses is the percent of protein mass in the main particle population. By cross-linking with bis-(sulfo succinimidyl)suberate these particles contain two apolipoproteins per particle. The original rHDL particles were incubated with LDL at 37 °C for 24 h.

The original rHDL particles were incubated with LDL and LCAT at 37 °C for 24 h.

*Only preparation shown by nonequilibriumondenaturating gradient gel electrophoresis to contain free apolipoprotein (17%).
Reconstituted Lipoproteins with ApoA-I, E, and A-IV

TABLE II
Spectral properties of rHDL particles

| rHDL | Apo  | PC/Apo | λ Maximum fluorescence* | Fluorescence intensity at maximum λ* | Fluorescence polarization* | α-Helix% |
|------|------|--------|--------------------------|--------------------------------------|---------------------------|---------|
| 1    | A-I  | 2.71/1 | 333                      | 642                                 | 0.090                      | 68      |
| 2    | A-I  | 1.35/1 | 334                      | 649                                 | 0.106                      | 62      |
|      | Free A-I |        | 335                      | 570                                 | 0.125                      | 50      |
| 3    | E    | 2.71/1 | 337                      | 590                                 | 0.091                      | 52      |
| 4    | E    | 1.35/1 | 337                      | 540                                 | 0.090                      | 46      |
|      | Free E |        | 340                      | 452                                 | 0.076                      | 38      |
| 5    | A-IV | 2.71/1 | 337 (shoulder), 348      | 324                                 | 0.129                      | 64      |
| 6    | A-IV | 1.35/1 | 337 (shoulder), 348      | 378                                 | 0.115                      | 52      |
|      | Free A-IV | | 337, 348 (shoulder)      | 180                                 | 0.097                      | 68      |

*The wavelengths of maximum fluorescence were obtained from uncorrected emission spectra, exciting at 280 nm and using 4-nm slits; the errors of measurement are ±1 nm. For the samples containing apoA-IV, the Tyr contribution to fluorescence emission appeared as a shoulder at 308 nm, and Trp spectra were bimodal, with peaks at 337 and 348 nm.

*Fluorescence polarization values were measured at 24 °C, exciting at 280 nm (4-nm slits); errors are ±0.003.

*Fluorescence polarization values were measured at 24 °C, exciting at 280 nm (4-nm slits); errors are ±0.003.

The % α-helix content was estimated from the expression of Chen et al. (27) and molar ellipticity values at 222 nm. The experimental errors are ±10%.

![Fig. 2. Denaturation behavior of free apoA-I (△), rHDL-1 (○), and rHDL-2 (□) containing apoA-I in weight ratios of 2.7/1 and 1.35/1, POPC/apoA-I, respectively. Panel A shows the change in the wavelength of maximum fluorescence of the apolipoprotein with increasing concentrations of guanidine hydrochloride. Panel B shows the change in the negative ellipticity at 222 nm (from CD spectra) of the apolipoprotein with increasing GdnHCl. The fluorescence and CD spectra were measured immediately after the sequential addition of solid GdnHCl, at 24 °C, using samples with an initial absorbance of 0.080 at 280 nm.](image)

![Fig. 3. Denaturation behavior of free apoE (△), rHDL-3 (○), and rHDL-4 (□) containing apoE in weight ratios of 2.7/1 and 1.35/1, POPC/apoE, respectively. Panels A and B present the same type of data as depicted in Fig. 2.](image)

![Fig. 4. Denaturation behavior of free apoA-IV (△), and rHDL-5 (○), and rHDL-6 (□) containing apoA-IV in weight ratios of 2.7/1 and 1.35/1, POPC/apoA-IV, respectively. Panels A and B contain the same type of data as depicted in Figs. 2 and 3. Since the Trp fluorescence spectra of apoA-IV were bimodal, the behavior of the peaks at 337 (△, ○) and 348 nm (△, □) was analyzed separately.](image)

**TABLE III**

Concentration of denaturant at 50% denaturation

| rHDL | Apo  | [GdnHCl] (from λ maximum fluorescence) | [GdnHCl] (from CD) |
|------|------|----------------------------------------|-------------------|
| 1    | A-I  | 5.40                                   | 2.80              |
| 2    | A-I  | 4.25                                   | 1.40              |
|      | Free A-I |                         | 1.25              |
| 3    | E    | 4.95                                   | 1.90              |
| 4    | E    | 3.75                                   | 1.65              |
|      | Free E |                         | 1.65              |
| 5    | A-IV | >5                                     | 2.30              |
| 6    | A-IV | >5                                     | 1.20              |
|      | Free A-IV |                        | 0.85              |

solvent at this denaturant concentration; this is particularly true of the rHDLs containing apoA-IV which show little or no denaturation by 5 M GdnHCl when measured by fluorescence wavelength shifts. Free apoE is the most stable of the free apolipoproteins, reflecting the presence of the stable N-terminal domain reported by Wetterau et al. (34). Free apoA-
IV is least stable when free in solution, in agreement with previous reports (33, 35), but increases markedly in stability in the lipid complexes. The stability of the apolipoproteins in rHDL particles increases with the content of lipid relative to apolipoprotein, and the multiple phases in the denaturation behavior for the apoA-I rHDL, and a little less so for the other complexes, suggest the presence of distinct structural domains in the apolipoproteins present in the rHDL particles.

**Reaction with LCAT**—Linear Lineweaver-Burk plots were obtained from kinetic experiments that measured the initial velocity of the LCAT reaction as a function of apolipoprotein concentration in the rHDL. Linear regression analysis of these data gave correlation coefficients in the range from 0.95 to 1.00, and the apparent kinetic parameters listed in Table IV. In general, the preparations with smaller average sizes, containing the same apolipoprotein, have a larger apparent \( V_{\text{max}} \), but also considerably larger apparent \( K_m \), values that result in smaller apparent \( V_{\text{max}}/K_m \), specificity constants. As indicated in Table IV, the larger complexes are from 3.4- to 8.5-fold more reactive than the smaller ones. In the case of apoA-I containing rHDL, the difference can be attributed to the different folding of apoA-I in the rHDL of different sizes which affects the activation of the LCAT reaction (2, 6). For the other complexes it is also possible that apolipoprotein structure plays a role in determining the reactivity with LCAT. In any case, within the class of particles with two apolipoproteins, the reactivity with LCAT varies considerably depending on the sizes of particles that are present.

The relative reactivity for the larger apoA-I, apoE, and apoA-IV complexes is 100, 19, and 23%, respectively, comparable to values reported previously (19, 20).

**DISCUSSION**

We showed in this study that discrete rHDL particles of reproducible sizes are formed with POPC and all three apolipoproteins. For the same protein and lipid, the average sizes of the particles are larger for the apolipoproteins with higher molecular weight; also, the particle sizes increase with the proportion of lipid to protein. In the case of the discoidal rHDL particles containing apoA-I and POPC, we have isolated previously several of these particles in pure form and have studied their structures and functional properties in detail (2, 5, 6). We have shown that the class of particles containing two apoA-I molecules comprise three main particle subclasses with diameters of 78, 87, and 94 Å, which have distinct apoA-I structures as revealed by fluorescence and CD spectroscopic measurements and by monoclonal antibody binding studies. Another class of apoA-I particles contains three molecules of apoA-I per particle, and consists of particle subclasses with apoA-I structures that resemble closely those of some subspecies in the 2 apoA-I class of particles. For example, the 78-Å particle in the 2 apoA-I class is very similar to the 108-Å particle in the 3 apoA-I class. These observations led us to propose a model for these discoidal rHDL particles where the subclasses of particles within each size class differ from one another by the number of \( \alpha \)-helical segments of the apoA-I that are in contact with lipid (2). Since the structure of the gene for apoA-I specifies up to eight \( \alpha \)-helical amino acid repeated sequences that coincide with potentially lipid-binding amphipathic \( \alpha \)-helical segments (11, 12), we proposed that these \( \alpha \)-helical segments become organized in an antiparallel, close-packed structure that binds to the lipid acyl chains and defines the disc diameter. The experimentally observed 78-, 87-, and 94-Å particles were consistent in size, composition, and apoA-I structure with discs containing 2 apoA-I molecules, each with 6, 7, or 8 \( \alpha \)-helical segments bound to lipid, respectively, as indicated in Fig. 5.

Since apoE and apoA-IV are similar to apoA-I in their gene structure and the presence of homologous 22-amino acid repeated sequences (11, 12): apoE with a maximum of 9 and apoA-IV with a maximum of 13, analysis of their particle sizes with POPC should provide a good test of our model. The original rHDL preparations with all three apolipoproteins were shown to contain two apolipoproteins per particle. Within this class of particles both apoE and apoA-IV formed distinct subclasses with diameters of 87 and 108 Å, and 116 and 145 Å, respectively. Assuming that the diameter of each \( \alpha \)-helical segment at the periphery of the disc is 15–16 Å, we calculate that these particles would contain 7 and 9, and 10 and 13 \( \alpha \)-helical segments per apolipoprotein, respectively (see Fig. 5). The largest particle sizes in the class with two apolipoproteins correlate perfectly with the maximum number of putative \( \alpha \)-helical segments found in the sequence of each apolipoprotein, rather than with their molecular weights. In addition, the ability to form smaller subclasses of particles probably with reduced numbers of \( \alpha \)-helices in contact with lipid, indicates that, as in the case of apoA-I, apoE and apoA-IV have considerable conformational flexibility that allows these apolipoproteins to carry more or less lipid, and to adapt to surfaces with different curvatures.

The rHDL particles containing apoA-I have been shown by us to undergo structural rearrangements between particle subclasses as phospholipid is depleted, either by spontaneous transfers or by transfers catalyzed by lipid transfer protein to LDL (29, 30). We have shown that upon loss of POPC 94-Å

**Table IV**

*Apparent (App.) kinetic constants for the reaction of LCAT with the rHDL.*

| rHDL   | Apo | PC/Apo | \( r \) | App. \( V_{\text{max}} \) | App. \( K_m \) | \( V_{\text{max}} \)/App. \( K_m \) | Relative reactivity of rHDL pairs |
|--------|-----|--------|--------|----------------|---------------|----------------|-------------------------------|
| 1      | A-I | 2.71/1 | 0.999  | 0.802          | 2.17 \times 10^7 | 3.70 \times 10^5 | 3.4                           |
| 2      | A-I | 1.35/1 | 0.969  | 0.722          | 6.58 \times 10^7 | 1.10 \times 10^6 | 1.0                           |
| 3      | E   | 2.71/1 | 0.947  | 0.142          | 1.92 \times 10^7 | 0.798 \times 10^5 | 8.5                           |
| 4      | E   | 1.35/1 | 0.988  | 0.632          | 7.29 \times 10^7 | 0.087 \times 10^6 | 1.0                           |
| 5      | A-IV| 2.71/1 | 0.997  | 0.323          | 3.79 \times 10^7 | 0.851 \times 10^5 | 4.7                           |
| 6      | A-IV| 1.35/1 | 0.991  | 0.847          | 3.57 \times 10^7 | 0.181 \times 10^6 | 1.0                           |

The apparent kinetic constants were obtained from Lineweaver-Burk analysis of initial velocity versus apolipoprotein concentration data. Four apolipoprotein concentrations in the range from \( 0.3 \times 10^{-4} \) to \( 5.5 \times 10^{-4} \) M were used; \( r \) is the correlation coefficient from linear regression analysis; CE is the cholesterol ester produced in the LCAT reaction.
particles are converted to 87-Å intermediates and finally to 78- and 108-Å products. The latter two particles, especially the 78-Å species, are very stable under various conditions. Similarly, we found in this study that the original rHDL particles formed with apoE and apoA-IV, when incubated with LDL, undergo structural rearrangements to smaller particles, and in the case of apoA-IV rHDL, also to larger particles (154 Å). The apoA-IV rHDL products (108 and 154 Å) are very likely counterparts of the 78- and 108-Å particles seen with the apoA-I particles, which contain 2 and 3 apoA-I per particle and 6 α-helical segments per apoA-I. The apoA-IV particles probably also contain 2 and 3 apolipoprotein molecules, but with 9 α-helical segments per apoA-IV. The apoE rHDL particles exposed to LDL change less than the apoA-I and apoA-IV containing particles; nevertheless, there is a shift in particle distribution towards the smaller 87-Å particle species. These experimental results further support the view that these apolipoproteins have multiple discrete structures that can adapt to the lipid content and surface of the particles.

Simultaneous incubation of the rHDL particles with LDL and LCAT produces distinct particle populations. The apoA-I particles of 93 Å have been characterized in detail by us in a previous study (6): they are spherical particles with a core of cholesterol ester, 3 apoA-I molecules per particle, and an apolipoprotein structure similar to the disc with 8 α-helices per apoA-I. The 76-Å species probably are similar to the reconstitution product described by Ritter and Scapin (31), with 2 apoA-I molecules per particle. The 108-Å shoulder probably represents intermediates in the conversion from discoidal to spherical rHDL. Clearly, the original discoidal rHDL with apoE and apoA-IV react effectively with LCAT to give discrete particles. These particles are very likely spherical and cholesterol ester rich, as demonstrated by Nichols and colleagues (36) in their studies of larger apoE containing rHDL discs reacted with LCAT. Unfortunately, we were not able to analyze the composition and the number of apolipoprotein molecules per particle because of the limited amount of samples, but assuming 3 apolipoproteins per particle, by analogy with the apoA-I containing spherical particles, the

- 93-, 104-, 113-, and 134-Å diameters are consistent with the presence of 8, 9, 10, and 12 α-helical segments per apolipoprotein, provided that these secondary structural elements retain their antiparallel and closely packed organization on the surface of the spheres (6).

The spectral properties of the free apolipoproteins indicate relatively high contents of α-helical structure and low stability to denaturation, in agreement with the results of other laboratories (34, 35, 37, 38). The α-helix contents of apoA-IV in solution, reported in the literature, range from 35 to 75% (38, 39) compared to 68% in this study. No doubt these discrepancies reflect the different preparation and handling procedures that result in different self-association and unstable structural states of apoA-IV in solution. Furthermore, dimerization of free apoA-IV has been reported by Weinberg and Spector (32) to occur at concentrations above 0.14 mg/ml, which is consistent with our observations of high α-helical content, bimodal Trp fluorescence emission spectra, and the predominantly dimeric form cross-linked with bis-(sulfosuccinimidyl)suberate for free apoA-IV at concentrations from 0.15 to 0.3 mg/ml. Cross-linked apoE appears tetrameric as reported by others (17, 37).

Incorporation of the apolipoproteins into rHDL complexes is accompanied, for apoA-I and apoE, by increases in α-helix content, small blue shifts in the wavelength of maximum fluorescence, and small increases in quantum yield. These are hallmarks for the development of amphipathic α-helical structure in contact with lipid and exposure of nonpolar amino acid residues, including Trp, to lipid (4, 40). Concomitantly, the stability to denaturation by GdnHCl increases markedly and is accentuated for the complexes with the higher content of lipids. The behavior of the rHDL particles containing apoA-IV is similar to that of the other two types of rHDL, in that the stability of the protein to denaturation increases dramatically when apoA-IV binds to lipid; however, the structural rearrangements of the protein going from the free form to the lipid-bound state entail a decrease in total α-helix content and a change in Trp environment that doesn’t seem to involve contact with lipid, yet is extremely stable towards denaturation as seen in Fig. 4. Furthermore, because it has a bimodal fluorescence spectrum, this single Trp residue (Trp39) of apoA-IV is either present in nonequivalent environments on the same particle or is present in two populations of particles with distinct properties. The first explanation is more tenable because a similar spectrum is observed for the free apoA-IV and both rHDL preparations containing apoA-IV in different particle populations.

Perhaps the most significant new result from the spectral measurements is the observation that for all three apolipoproteins the two rHDL preparations with different PC/apolipoprotein ratios and particle distributions have two apolipoprotein molecules per particle, yet demonstrate distinct spectral properties (i.e. distinct apolipoprotein structures). Therefore, it can be concluded that apoE and apoA-IV, like apoA-I, can adopt different conformations in lipid bound states.

The function of these distinct structural states of the apolipoproteins also varies as indicated by the kinetics of the reaction with LCAT (Table IV). The larger subclasses of particles are significantly more reactive than the smaller ones. In the case of the rHDL particles containing apoA-I, we have proposed that the region of apoA-I around the Lys107 residue rearranges during the interconversion of the 96-Å particles to the 78-Å species (41). Thus, we suggest that this region is also involved in the dramatic difference in the reactivity towards LCAT demonstrated by the pure 96- and 78-Å particles (2.

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2 K. H. Toohill and A. Jonas, unpublished results.
6). It is possible that similar structural rearrangements, involving α-helical segments in the apoE and apoA-IV sequences, modulate the activation of the LCAT reaction by these apolipoproteins.

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