Altering in Apolipoprotein A-I 22-Mer Repeat Order Results in a Decrease in Lecithin:Cholesterol Acyltransferase Reactivity*

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Apolipoprotein A-I contains eight 22-amino acid and two 11-amino acid tandem repeats that comprise 80% of the mature protein. These repeating units are believed to be the basic motif responsible for lipid binding and lecithin:cholesterol acyltransferase (LCAT) activation. Computer analysis indicates that despite a fairly high degree of compositional similarity among the tandem repeats, significant differences in hydrophobic and amphipathic character exist. Our previous studies demonstrated that deletion of repeat 6 (143–164) or repeat 7 (165–186) resulted in a 98–99% reduction of LCAT activation as compared with wild-type apoA-I. To determine the effects of substituting one of these repeats with a more hydrophobic repeat we constructed a mutant apoA-I protein in which residues 143–164 (repeat 6) were replaced with repeat 10 (residues 220–241). The cloned mutant protein, 10F6 apoA-I, was expressed and purified from an Sf-9 cell baculoviral system and then analyzed using a number of biophysical and biochemical techniques. Recombinant complexes prepared at a 100:5:1 molar ratio of L-α-dimyristoylphosphatidylcholine: cholesterol:wild-type or 10F6 apoA-I showed a doublet corresponding to Stokes diameters of 114 and 108 Å on nondenaturing 4–30% polyacrylamide gel electrophoresis. L-α-Dimyristoylphosphatidylcholine 10F6 apoA-I complexes had a 5–6-fold lower apparent \( V_{app} \) compared with wild-type apoA-I containing particles. As expected, monoclonal antibody epitope mapping of the lipid-free and lipid-bound 10F6 apoA-I confirmed that a domain expressed between residues 143 and 165 normally found in wild-type apoA-I was absent. The region between residues 119 and 144 in 10F6 apoA-I showed a marked reduction in monomeric antibody binding capacity. Therefore, we speculate that the 5–6-fold lower LCAT reactivity in 10F6 compared with wild-type apoA-I recombinant particles results from increased stabilization within the 121–165 amino acid domain due to more stable apoprotein helix-phospholipid interactions as well as from conformational alterations among adjacent amphipathic helices repeat.

There is a significant, negative correlation between the plasma concentration of high density lipoproteins (HDLs) and coronary artery disease in human populations and in animal models of atherosclerosis (1–3). Recent investigations using apolipoprotein A-I (apoA-I) transgenic and knockout animals have clearly demonstrated the protective role of HDLs in the prevention of atherosclerosis by "reverse cholesterol transport" (4–8). The predominant protein constituent of HDL, apoA-I, solubilizes and organizes phospholipid, cholesterol, and cholesteryl ester in plasma and facilitates the distribution of cholesteryl ester between hepatic and extrahepatic tissue. Conversion of nascent HDLs or discoidal complexes into mature HDL particles is mediated by the plasma enzyme lecithin:cholesterol acyltransferase (LCAT). When activated by apoA-I, LCAT catalyzes the conversion of HDL-cholesterol to HDL-cholesteryl esters (4, 5). However, the precise mechanism of LCAT activation by HDL-bound apoA-I remains largely unknown.

Investigations aimed at identifying how apoA-I organizes lipid and activates LCAT have focused on the unique structural properties of this protein (6–14). ApoA-I contains 10 tandem repeats, each having substantial \( \alpha \)-helical character that typically begin with a proline residue, a feature that is shared within the entire apolipoprotein supergene family. These repeats comprise approximately 80% of the total protein and are divided into two 11-mer units and eight 22-mer units (15, 16), a feature that is highly conserved across species. When displayed on an Edmunson wheel, the \( \alpha \)-helices show a distinctive amphipathic character with separate hydrophilic and hydrophobic faces. The amphipathic nature of these repeating units is believed to be responsible for the ability of apoA-I to organize lipid and to activate LCAT (17). A number of different models have been suggested to explain how apoA-I's amphipathic repeats associate with phospholipid and how these repeats activate LCAT (6–14, 18–25). Progress toward obtaining crystals of either lipid-free or lipid-bound apoA-I has been slow, and a three-dimensional crystal structure is not available to reconcile the different models.

We have continued our investigation into the structure-function relationships of apoA-I by constructing a 22-mer substitution mutant in a region previously shown to be necessary for LCAT activation. Sequential deletion of any one of the 10 tandem repeats results in a modest (30%) or dramatic (99%) reduction in LCAT reactivity compared with wild-type apoA-I (25). However, several studies (26, 27) have shown that the region corresponding to residues 143–186 (22-mer, repeats 6 and 7) was absolutely essential for LCAT activation. In this paper, we report the properties of a mutant apoA-I protein in which repeat 6 (residues 143–164) was replaced with a copy of...
repeat 10 (residues 220–241). The cloned mutant protein, called 10F6 apoA-I, was expressed and purified from an SF-9 cell baculoviral system and used for biophysical characterization, monoclonal antibody epitope mapping, and LCAT reactivity measurements. In this report, we describe those structural features of the residue 121–186 domain that are important for optimal LCAT activation.

**EXPERIMENTAL PROCEDURES**

HPLC grade organic solvents were purchased from Fisher. All other chemicals, reagents were purchased from Sigma and other noted. [3H]Cholesterol was purchased from Du Pont NEN. Tissue culture reagents, restriction endonucleases, and other DNA modifying enzymes were purchased from Life Technologies, Inc.

Expression of Wild-type and Mutant pBlueBac III:ApoA-I Constructs—Wild-type and 10F6 apoA-I cDNA pBlueBac III constructs were used in conjunction with the Autographa californica nuclear polyhedrosis virus linear viral DNA for co-transfection into SF-9 cells, as described previously (28). Recombinant wild-type and mutant baculoviral clones were purified and used for the generation of high titer viral stocks as described previously (28). To prevent degradation of the expressed protein, pepstatin A and leupeptin (at a final concentration of 700 μg/ml and 500 μg/ml) were added to the culture medium 36 h post-infection.

Preparation and Purification of Recombinant Wild-type and 10F6 ApoA-I—Preparation and purification of recombinant wild-type and 10F6 apoA-I protein were carried out as previously reported (28). Briefly, at the time of harvest (50–72 h), SF-9 medium was spun at 4°C, 10,000 rpm for 10 min, and the supernatant was adjusted to 10% NaCl, 0.25 mM EDTA, and 0.15 mM sodium azide at 39°C. Recombinant wild-type and mutant baculoviral clones were purified and used for the generation of high titer viral stocks as described previously (28). Briefly, at the time of harvest (50–72 h), SF-9 medium was spun at 4°C, 10,000 rpm for 10 min, and the supernatant was adjusted to 10% NaCl, 0.25 mM EDTA, and 0.15 mM sodium azide, 0.6% fatty acid-free bovine serum albumin, 2 mM M-mercaptoethanol, and 25 ng of purified human plasma LCAT (kindly provided by Dr. J. S. Parks). The reactions were carried out for 60 min at 37°C, and the conversion of [3H]cholesterol to cholesteryl ester was kept below 15% to maintain first order kinetics. Background values were determined by omitting LCAT from the reaction tube. The fractional cholesteryl esterification rate was multiplied by the nmol of substrate cholesterol in the assay tube, corrected for the background, and converted to nmol of cholesterol ester formed/h of LCAT. Apparent V \( V_{\text{max}} \) and K \( K_c \) values were determined from Hanes-Woolf plots (34) of discoidal cholesterol substrate concentration (μM) divided by the cholesteryl ester formation rate (nmol/h/ml of LCAT) versus discoidal cholesterol concentration (μM). The activation energy (E*) for cholesteryl ester formation was derived from Arrhenius plots of the LCAT reactivity of wild-type and 10F6 discoidal substrates. Reactions were performed at 37, 34, 31, 28, and 25°C using 0.55 μg of discoidal cholesterol substrate and 25 ng of purified human LCAT. Arrhenius plots were constructed as the reciprocal of incubation temperature in Kelvin versus the log of cholesteryl ester formed (nmol/h) (34). Linear regression analysis was used to determine the activation energy according to the formula, E* (cal/mol) = −2.33R (slope), where R is the gas constant (1.987 cal deg⁻¹ mol⁻¹).

**RESULTS**

To test whether substitution of a single 22-mer repeat located at 143–164 (repeat 6) would alter apoA-I's ability to bind lipid or to activate LCAT, we repeated repeat 6 of wild-type apoA-I with repeat 10 (corresponding to amino acids 220–241). The boxed region in Fig. 1 shows the primary amino acid sequence of wild-type and 10F6 apoA-I within the residue 143–164 domain. The mutant protein 10F6 apoA-I contains one molecular weight of the protein, \( \lambda \) is the optical path length in centimeters, and \( c \) is the protein concentration in g/ml. The percentage of a-helix was calculated from the formula of Chen et al. (32), \( \operatorname{a-helix} = -30,300f_2 - 2,340 \). Stability of the discoidal 10F6 and wild-type DMPC complexes was determined by plotting the mean residue ellipticity versus guanidine HCl concentration as expressed as the concentration of guanidine HCl (\( D_25 \)) that reduced the ellipticity by 50%.

Epitope Mapping Studies—Competitive solid phase immunoassays were used to assess the binding of monoclonal antibodies to lipid-free apoA-I and recombinant DMPC phospholipid complexes containing apoA-I. With two exceptions (antibodies AI-17 and AI-141.7), each antibody has been described, and its epitope on wild-type apoA-I have been documented (32). The epitopes of antibodies AI-17 and AI-141.7 were localized to residues 143–165 and 220–242, respectively, on the basis of their binding to numerous apoA-I mutant proteins described previously. The immunoassays were performed as described (33). Briefly, isolated human plasma apoA-I or plasma HDL (0.05 mL of 5 μg/ml) was immobilized onto 96-well Falcon 3911 Microtiter HI flexible assay plates. After coating the plates, increasing amounts of purified apoA-I or recombinant A-I discs (0.025 ml) diluted in phosphate-buffered saline containing 3% normal goat serum were added to wells in the presence of 0.025 ml of ascites fluid containing a limiting amount of monoclonal antibody (typically dilutions of 10⁻¹ to 10⁻⁶). Competitor concentrations listed in the figures represent the final concentration in the 0.05-ml reaction volume. Plates were incubated overnight at 4°C. After washing the wells, mouse antibody binding to the immobilized antigen was detected by a second 1-h incubation at 37°C with 125-I-labeled goat anti-mouse IgG. All data were expressed as B/B₀ where B is the cpm bound in the presence of competitor, and B₀ is the cpm bound in the absence of competitor. To compare the affinity of the antibodies for DMPC discs containing wild-type or mutant apoA-I’s competitor, the slopes of log-transformed B/B₀ ratios were obtained by linear regression and subjected to tests of equality.

**LCAT Reaction Kinetics**—The LCAT reaction was monitored by following the cholesterol to cholesteryl ester conversion using recombinant discoidal complexes containing either wild-type or 10F6 apoA-I. The complexes were using 0.15 μg of substrate cholesterol in a final concentration of 10 μM Tris, pH 7.4, 140 mM NaCl, 0.25 mM EDTA, and 0.15 mM sodium azide, 0.6% fatty acid-free bovine serum albumin, 2 mM β-mercaptoethanol, and 25 ng of purified human plasma LCAT (kindly provided by Dr. J. S. Parks). The reactions were carried out for 60 min at 37°C, and the conversion of [3H]cholesterol to [3H]cholesteryl ester was determined by lipid extraction of the incubation mixture followed by thin layer chromatography (28). The extent of cholesterol esterification was kept below 15% to maintain first order kinetics. Background values were determined by omitting LCAT from the reaction tube. The fractional cholesteryl esterification rate was multiplied by the nmol of substrate cholesterol in the assay tube, corrected for the background, and converted to nmol of cholesterol ester formed/h of LCAT. Apparent V \( V_{\text{max}} \) and K \( K_c \) values were determined from Hanes-Woolf plots (34) of discoidal cholesterol substrate concentration (μM) divided by the cholesteryl ester formation rate (nmol/h/ml of LCAT) versus discoidal cholesterol concentration (μM).

The activation energy (E*) for cholesteryl ester formation was derived from Arrhenius plots of the LCAT reactivity of wild-type and 10F6 discoidal substrates. Reactions were performed at 37, 34, 31, 28, and 25°C using 0.55 μg of discoidal cholesterol substrate and 25 ng of purified human LCAT. Arrhenius plots were constructed as the reciprocal of incubation temperature in Kelvin versus the log of cholesteryl ester formed (nmol/h) (34). Linear regression analysis was used to determine the activation energy according to the formula, E* (cal/mol) = −2.3R (slope), where R is the gas constant (1.987 cal deg⁻¹ mol⁻¹).

**Data Analysis**—Values are given as the mean ± S.D. Statistical comparisons were made using Student’s t test. L. Curtis and M. G. Sorci-Thomas, unpublished observations.
copy of repeat 10 inserted in the region normally occupied by repeat 6 and another copy of repeat 10 in its native position, 220–241. Also shown in Fig. 1 is the calculated hydrophobic moment $\langle \mu H \rangle$ and the average hydrophobicity ($\langle H \rangle$) for the entire boxed 22-mer amphipathic helix. The calculated values, $\langle \mu H \rangle$ and $\langle H \rangle$, for this region in wild type and 10F6 are $\langle \mu H \rangle = 0.37$, $H = -0.30$ and $\langle \mu H \rangle = 0.24$, $H = +0.24$, respectively (35, 36). Replacing repeat 6 with repeat 10 increases thehydrophobicity of the 143–164 region in 10F6 apoA-I from $-0.30$ to $+0.24$, reflecting the greater proportion of nonpolar and uncharged amino acids in this 22-mer.

Recombinant HDL complexes were prepared using a molar ratio of 100:5:1, DMPC:cholesterol:apoA-I, respectively. Fig. 2 shows a Coomassie-stained 4–30% nondenaturing gradient gel of 10F6, wild-type, and plasma apoA-I containing recombinant discoidal complexes. The gel shows doublets corresponding to Stokes diameters of 114 and 108 Å for each of the recombinant apoA-I complexes prepared. The final DMPC:cholesterol:apoA-I ratio of the three discoidal preparations was 98.1:4.1:1, 90.7:4.8:1, and 104:4.0:1 for 10F6, wild-type, and plasma apoA-I-containing particles, respectively.

The effect of the 10F6 apoA-I replacement mutation on the overall secondary structure of wild-type apoA-I protein was first examined by determining the percentage of $\alpha$-helix content. Both lipid-free and lipid-bound wild-type and 10F6 apoA-I were analyzed by circular dichroism spectroscopy. As shown in Table I, a trend toward a higher $\alpha$-helix content for both the lipid-free and lipid-bound 10F6 apoA-I compared with wild-type apoA-I was noted. However, these differences did not reach statistical significance at the level of $p < 0.05$. When examined in the lipid-bound state, the $\alpha$-helix content for each of the apoA-IIs studied increased compared to its lipid-free state (Table I).

Although the 10F6 apoA-I substitution did not show a statistically significant change in its $\alpha$-helix content compared with lipid-free or lipid-bound wild-type apoA-I, the DMPC 10F6 apoA-I recombinant complexes were found to be less susceptible to guanidine HCl denaturation than DMPC wild-type apoA-I complexes. Guanidine HCl denaturation of DMPC 10F6 apoA-I complexes gave a $D_{280} = 2.8$ M, while DMPC wild-type complexes gave a $D_{280} = 2.1$ M (data not shown). These results demonstrate that the lipid-protein interaction in DMPC 10F6 complexes was more stable than that for DMPC wild-type apoA-I complexes.

In the next set of experiments the effects of substituting apoA-I’s repeat 6 with repeat 10 were evaluated with respect to the protein’s ability to activate LCAT. Kinetic studies were conducted, and the $K_m$ and $V_{max}$ of the reactions are summarized in Table II. The apparent $K_m$ values are 1.2 and 6.0 μM and the apparent $V_{max}$ values are 51.0 and 48.0 nmol/ml/h for DMPC wild-type and 10F6 apoA-I complexes, respectively (Table II). The overall efficiency for the LCAT reaction, referred to as the apparent $V_{max}/K_m$, was calculated to be 42.5 and 8.0 nmol/ml/h/μM for DMPC wild-type and 10F6 apoA-I complexes, respectively. To probe the mechanistic basis for the lower LCAT efficiency observed with the 10F6 apoA-I-containing particles, the energy of activation was measured as a function of temperature. The Arrhenius plots shown in Fig. 3 produced activation energies of 16.0 and 25.3 kcal/mol for DMPC wild-type and 10F6 apoA-I complexes, respectively.

To further explore the conformational impact of the 22-mer replacement mutant on apoA-I secondary structure, epitope mapping studies were carried out using a panel of defined monoclonal antibodies (33). First, the binding capacity of each antibody for lipid-free wild-type or 10F6 apoA-I was compared in competitive immunoassays. The results are tabulated in Table III. Six antibodies that identify N-terminal epitopes between residues 1 and 126 and three antibodies that identify C-terminal epitopes between residues 178 and 242 bound well to both apoproteins. These antibodies gave binding ratios (10F6 to wild type) of 5 or less, indicating that these particular epitopes were expressed by both apoproteins and that there were only minimal differences in the extent of epitope expression between the two apoA-I proteins. In contrast, four antibodies that identify epitopes between residues 119 and 165 on wild-type apoA-I either did not bind to purified 10F6 apoA-I or

### Table I

| Without DMPC | With DMPC |
|--------------|-----------|
| Wild-type apoA-I | 42.8 ± 7.1 | 67.2 ± 4.0 |
| Plasma apoA-I | 47.0 ± 7.8 | 65.3 ± 8.7 |
| 10F6 apoA-I | 49.1 ± 4.8 | 72.5 ± 4.4 |

%helix content of wild-type and 10F6 apoA-I

Values were determined from molar ellipticities at 222 nm. All values represent the mean ± S.D. of four determinations.

### Table II

| | Without DMPC | With DMPC |
|---|---|---|
| Wild-type apoA-I | 1.2 | 6.0 |
| Plasma apoA-I | 51.0 | 48.0 |
| 10F6 apoA-I | 42.5 | 8.0 |

| Stokes Radius (nm) | HMW | 10F6 | Wild-type | plasma |
|---|---|---|---|---|
| 8.5 nm | 6.1 nm | 4.6 nm | 4.1 nm |

Distribution of the 143–164 Domain

of Wild-Type and 10F6 Apo A-I

| Wild-type Apo A-I | 140 | 145 | 150 | 155 | 160 | 165 |
|---|---|---|---|---|---|---|
| QEKLSPLGEEMRDARHVDALRTHLAPT | $\mu H = 0.37$ | $H = -0.30$ |
| 10F6 Apo A-I | 140 | 145 | 150 | 155 | 160 | 165 |
| QEKLSPLTVLSSFKVSFLSALEETYTKLNP T | $\mu H = 0.24$ | $H = +0.24$ |

### Fig. 1

Sequence comparison of the residue 143–164 domain of wild-type and 10F6 apoA-I. The boxed 22-mer for wild-type apoA-I shows the amino acid sequence normally found in repeat 6 (residues 143–165). The boxed 22-mer for 10F6 apoA-I shows the amino acid sequence that corresponds to repeat 10 or residues 220–241. The calculated hydrophobic moment ($\langle \mu H \rangle$) and the average hydrophobicity ($\langle H \rangle$) for the entire boxed 22-mer amphipathic helix are shown for wild-type and 10F6 apoA-I.

### Fig. 2

Coomassie G-250-stained 4–30% nondenaturing polyacrylamide gradient gel of DMPC cholesterol apoA-I discoidal complexes. HMW lane, calibrating high molecular weight standards and their corresponding Stokes radii: thyroglobulin (8.5 nm), ferritin (6.1 nm), catalase (4.6 nm), lactate dehydrogenase (4.1 nm). 10F6 lane, DMPC 10F6 apoA-I complexes. Wild-type lane, DMPC wild-type apoA-I complexes. Plasma lane, DMPC human plasma-derived apoA-I complexes. Each lane of recombinant particles contained 6 μg of protein. All recombinant discoidal preparations were prepared as described under “Experimental Procedures.”
bound very poorly, i.e. gave 10F6 to wild type binding ratios of 16 or greater (Table III).

To probe the conformational changes caused by association of apoA-I with lipid, we studied the binding of the same 13 antibodies to the recombinant DMPC discoidal complexes containing wild-type or 10F6 apoA-I. The six N-terminal antibodies, AI-16, AI-1.2, AI-19.2, AI-11, AI-4, and AI-115.1, had comparable affinity for both apoprotein-lipid complexes and had 10F6 to wild-type apoA-I binding ratios of 1.1 or less. This indicates that each of these epitopes was fully expressed on both the wild-type and 10F6 apoA-I when complexed with lipid. Competitive binding curves for two of these six antibodies are shown in Fig. 4. These antibodies bind epitopes that are linearly adjacent to helix 6, the AI-4 epitope within residues 99–121 and the AI-115.1 within residues 115–126. The superimposable curves confirm that AI-4 has both comparable binding capacity and binding affinity for the DMPC wild-type and 10F6 complexes. The displaced binding curves indicates that antibody AI-4 has both comparable binding affinity for the DMPC wild-type and 10F6 apoA-I complexes. However, the similar slopes of the two AI-115.1 curves demonstrates that antibody AI-115.1 binds the two epitopes with comparable affinity (Fig. 4). The three C-terminal antibodies, AI-178.1, AI-187.1, and AI-144, also bound both lipid-associated apoproteins with ratios between 1 and 7, and this pattern of reactivity was consistent with the results obtained with the lipid-free apoproteins (data not shown). Antibodies AI-137.1 and AI-17, which did not bind the lipid-free 10F6 apoA-I, also did not bind to the DMPC 10F6 apoA-I (Fig. 5), although they bound the wild-type apoA-I complexes. These data are in complete agreement with those obtained with the lipid-free apoA-Is (Table III) and further confirm the absence of these epitopes within the 137–165 region of 10F6 apoA-I protein. Competition curves for the two antibodies that bound residues 119–144, a region that both adjoins and slightly overlaps the deleted repeat 6 (residues 143–164), are shown in Fig. 6. These antibodies needed significantly higher concentrations of lipid-free 10F6 apoA-I to achieve 50% inhibition of binding (Fig. 6, top panels), as compared with wild-type apoA-I. These results strongly suggest that replacement of repeat 6 with repeat 10 changed the conformation of epitopes in repeat 5 (residues 121–142) but had little effect on the conformation of epitopes in repeat 7. However, these same repeat 5 epitopes were more similar when the apoA-Is were complexed with DMPC (Fig. 6, bottom panel). Taken together, these results imply that repeats 5 and 6 of apoA-I interact with phospholipid in a manner that normalizes and stabilizes a native conformation at the lipid interface.

**DISCUSSION**

In this report we describe the biophysical and biochemical properties of the structural mutant, 10F6 apoA-I. This mutant protein was designed to investigate the effect of a single 22-mer repeat substitution on apoA-I structure and function. In previous studies (25), we showed that deletion of repeat 6 (residues 143–164) or repeat 7 (165–186) reduces the overall LCAT reaction velocity by 98–99% relative to wild-type apoA-I (25). From these observations and from those of others (26, 27), we proposed that the reduced LCAT reactivity observed with the 36 apoA-I 22-mer deletion mutants resulted from disruption of interactions between adjacent helical repeats within apoA-I. We also suggested that electrostatic and hydrophobic side chain interactions were responsible for the proper alignment of the phospholipid substrate for LCAT catalysis (25). In the present studies, we have further refined our working model by characterizing apoprotein helix-lipid interactions and the apoprotein helix-helix interactions that are critical for optimal LCAT reactivity.

Recombinant DMPC complexes containing 10F6 apoA-I displayed a particle size distribution that was similar to wild-type and human plasma apoA-I-containing complexes. These data...
that the overall catalytic efficiency (apparent $K_m$) of the LCAT reaction as a function of cholesterol concentration.

The kinetic parameters are shown in Table II and demonstrate that the reactivity of the DMPC 10F6 apoA-I 22-mer repeat within the 143–165 domain resulted in a sub-

stantial reduction in LCAT binding to the recombinant substrate and ultimately in a lower catalytic efficiency. The decrease in LCAT catalytic efficiency was accompanied by a 9 kcal/mol increase in the activation energy for 10F6 compared with wild-type apoA-I recombinant complexes (Fig. 3). This increase in activation energy for catalysis of DMPC 10F6 complexes may reflect the interaction of hydrophobic residues (residue 143–165 domain) with boundary phospholipid, in addition to altering side chain interactions between adjacent helices in this region (repeats 5, 6, and 7). This hypothesis is strengthened by the guanidine denaturation studies in which DMPC 10F6 complexes were denatured at a higher molar concentration of guanidine hydrochloride (2.8M) than DMPC wild-type complexes (2.1M). The carboxyl-terminal region or repeats 9 and 10 (residues 209–243) of apoA-I has been proposed to be important in cell metabolism of HDL (40, 42). Recent studies using truncation mutants of the carboxyl-terminal region demonstrated that the domain corresponding to residues 227–243 was critical in modulating the association of apoA-I with lipoproteins as well as in the in vivo metabolism of HDL (40, 42). Other investigations show that substitution of the carboxyl-terminal region of apoA-I with a helical domain derived from apoA-II increases its hydrophobicity but does not restore the mutant protein’s lipoprotein affinity or its ability to associate with HDL$_3$ (42). It is possible that in vivo apoA-I’s carboxyl-terminal domain interacts cooperatively with the amphipathic helices located in the middle of the protein, thus allowing a conformation that can conform to particles of varying size and composition (42). The mechanistic role of this region in LCAT activation appears to be secondary to its role in lipid-lipoprotein association. Several studies have shown that deletion of repeat 9 or 10 results in at least a 60–90% reduction in wild-type LCAT activation (25–27, 41), while in one study, truncation of the residue 193–243 domain resulted in a 245% increase in wild-type LCAT activation (41). Thus, although this...
region may play some role in LCAT activation, it appears that the contribution of the carboxyl-terminal region of apoA-I to LCAT activation is secondary to its intrinsic lipid binding affinity and particle formation properties.

Protein-lipid hydrophobic interactions are believed to play a critical role in the activation of LCAT. Because LCAT is a surface active protein that binds to the discoidal lipid-protein interface, it has been suggested that apoA-I's amphipathic α-helices serve to disrupt the aqueous to lipid interface and expose phospholipid to LCAT (4, 5). Recently, NMR studies using an apoA-I fragment, residues 166–185 (12), as well as a synthetic peptide activator of LCAT, i.e. LAP-20, have shown that hydrophobic interactions between the nonpolar amino acids and the phospholipid acyl chains play an important role in stabilizing lipid-protein complexes (43). Furthermore, the NMR studies suggest that intermolecular salt bridges and “snorkeling” (44) of basic amino acid side chains play a less important role compared with hydrophobic interactions in stabilizing lipid-apoprotein interactions (43). Recent studies using synthetic peptides corresponding to each of the eight 22-mer repeats of apoA-I have shown that repeat 10 (220–241) has the highest lipid binding affinity of all eight repeats (45) and the greatest calculated depth of penetration into phospholipid vesicles (11). However, the relative lipid binding affinity of an amphipathic helix does not directly relate to a peptides' ability to activate LCAT (13).

Modeling studies have suggested that the mode of assembly of adjacent amphipathic helical repeats around the edge of a discoidal complex is determined by both the hydrophobic character of the residues and by the charge complementary along the edge of the helices (46). From crystallography studies, helix-helix interactions inside lipid bilayers have been shown to include interhelical salt bridges, hydrogen bonds, or precise packing interactions (47). Thus, these structural features may determine the overall stability and the relative orientation of the adjacent helices. Therefore, we suggest from our data that substitution of repeat 10 for repeat 6 alters the expression of at least one repeat 5 epitope, repeat remained unchanged. However, the substitution of repeat 6 altered the expression of at least one repeat 5 epitope, repeat 6 epitopes defined by antibodies AI-115.1 and AI-141.7 (residues 220–242), greater reactivity of antibodies AI-137.1 and AI-17 were lost. Finally, the three antibodies that identified epitopes that were C-terminal to repeat 6 reacted similarly with both lipid-free and lipid-bound wild-type and 10F6 apoA-I. Although the 10F6 apoA-I protein contains two copies per molecule of the AI-141.7 epitope (residues 220–242), greater reactivity of antibody AI-141.7 for this mutant apoA-I was not observed with either lipid-free or lipid-bound 10F6 apoA-I. Overall, these results suggest that substitution of repeat 6 for repeat 10 resulted in alteration of native wild-type apoA-I epitopes in both repeats 5 and 6 (residues 121–165). The conformational alterations in repeats 5 and 6 were found to become less apparent when the apoprotein was complexed with lipid, presumably as a result of lipid-apoprotein interactions that dominated apoA-I's helix-helix interactions. In summary, these studies have aided our understanding of the mechanism of apoA-I's activation of LCAT by clarifying the nature of the interaction between phospholipid and apoA-I's amphipathic α-helices and the intermolecular helix-helix interactions critical for optimal catalytic efficiency.

Acknowledgments—We recognize the excellent technical assistance of Neil Nordin, Kathi Richards, Gregory Pate, Abraham Gebre, and Elizabeth Eagleston. We thank Dr. Lewis Nelson for helpful discussions and Dr. Larry Rudel for support throughout this project.

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