Interaction of an Exchangeable Apolipoprotein with Phospholipid Vesicles and Lipoprotein Particles

ROLE OF LEUCINES 32, 34, AND 95 IN LOCUSTA MIGRATORIA APOLIPOPHORIN III*

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Apolipoprotein III (apoLp-III) from Locusta migratoria is an exchangeable apolipoprotein that binds reversibly to lipid surfaces. In the lipid-free state this 164-residue protein exists as a bundle of five elongated amphipathic α-helices. Upon lipid binding, apoLp-III undergoes a significant conformational change, resulting in exposure of its hydrophobic interior to the lipid environment. On the basis of x-ray crystallographic data (Breiter, D. R., Kanost, M. R., Benning, M. M., Wesenberg, G., Law, J. H., Wells, M. A., Rayment, I., and Holden, H. M. (1991) Biochemistry 30, 603–608), it was proposed that hydrophobic residues, present in loops that connect helices 1 and 2 (Leu-32 and Leu-34) and helices 3 and 4 (Leu-95), may function in initiation of lipid binding. To examine this hypothesis, mutant apoLp-IIIIs were designed within the three Leu residues were replaced by Arg, individually or together. Circular dichroism spectroscopy and temperature and guanidine hydrochloride denaturation studies showed that the mutations did not cause major changes in secondary structure content or stability. In lipid binding assays, addition of apoLp-III to phospholipid vesicles caused a rapid clearance of vesicle turbidity due to transformation to discoidal complexes. L34R and L32R/L34R/L95R apoLp-IIIIs displayed a much stronger interaction with lipid vesicles than wild-type apoLp-III. Furthermore, it was demonstrated that the mutant apoLp-IIIIs retained their ability to bind to lipoprotein particles. However, in lipoprotein competition binding assays, the mutants displayed an impaired ability to initiate a binding interaction when compared with wild-type apoLp-III. The data indicate that the loops connecting helices 1 and 2 and helices 3 and 4 are critical regions in the protein, contributing to recognition of hydrophobic defects on lipoprotein surfaces by apoLp-III.

Exchangeable apolipoproteins are important plasma proteins that bind reversibly to lipoproteins, a process triggered by an adaptation allows contact of hydrophobic faces of amphipathic α-helices with the lipid surface. Based on structural data, it has been proposed that apoLp-III recognizes potential lipid surface-binding sites via one of its ends (16) or the loops connecting the helices (7). The loops connecting helices 1 and 2 (loop A) and helices 3 and 4 (loop C) possess hydrophobic character (see Fig. 1). Sequence alignment of apoLp-IIIIs from different species indicates that the Leu residues in these loops are conserved (17). These data have led to the hypothesis that they function as a “hydrophobic sensor” (18). We have used site-directed mutagenesis to test this hypothesis by substituting hydrophobic residues in the putative “sensor” loops of L. migratoria apoLp-III. Characterization of lipid and lipoprotein binding properties of wild-type and mutant apoLp-IIIIs provide evidence that the Leu residues in loops A and C function in initiation of lipoprotein binding.

MATERIALS AND METHODS

Site-directed Mutagenesis—Site-directed mutagenesis was performed using the pALTER system (Promega, Madison, WI). The cDNA

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1 The abbreviations used are: apo, apolipoprotein; apoLp-III, apolipoplorin III; DMPC, dimyristoylphosphatidylcholine; DMPG, dimyristoylphosphatidylglycerol; LDL, low density lipoprotein; PL-C, phospholipase C; WT, wild-type.
for apoLp-III present in pET22b(+). A modified Contin program by Provencher and Gloc-IIIIs and to monitor temperature- or guanidine hydrochloride-induced denaturation (8). The resulting apoLp-III/pET construct was used as template DNA for site-directed mutagenesis reactions. Primers, designed to substitute Arg for Leu-32, Leu-34, or Leu-95, were added to alkali-denatured template DNA together with ampicillin repair and tetracycline knockout primers. All primers were phosphorylated. The annealing reaction was carried out at 75 °C for 5 min and cooled slowly to room temperature (~45 min). T4 DNA polymerase and ligase were added, and the incubation was continued for 90 min at 37 °C. High efficiency Escherichia coli cells were transformed with 2.5 μl of the reaction mix and grown overnight in LB medium with 125 μg/ml ampicillin. This culture was used for a plasmid miniprep, and the extracted DNA was used to transform JM109 cells. DNA was extracted from transformed JM109 cells and subsequently sequenced using the dideoxy nucleotide chain termination method (19) to verify the desired mutations. DNA from a positive clone was digested with EcoRI and XbaI, and the apoLp-III insert was subcloned back into the original pET22b(+) vector. Finally, the mutated apoLp-III/pET constructs were used to transform BL21 (DE3) E. coli cells.

Expression and Purification of ApoLp-III—ApoLp-III was expressed in E. coli BL21 cells harboring the apoLp-III/pET vector as described (10) and purified by reversed-phase high pressure liquid chromatography. The purity of the apoLp-III preparation was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and analytical reversed-phase high pressure liquid chromatography.

apoLp-III was obtained by growing E. coli harboring the apoLp-III/pET construct in 50 ml of M9 minimal medium at 37 °C. When the absorbance at 600 nm reached 0.6, apoLp-III expression was induced with isopropyl-β-D-thiogalactopyranoside (2 mM final concentration). The cells were further grown at 30 °C for 30 min after which 150 μCi of a tritiated amino acid mixture was added (Amersham Pharmacia Biotech, Oakville, Ontario, Canada). The cells were grown for another 4.5 h and pelleted by centrifugation. The cell-free supernatant, which is highly enriched in recombinant apoLp-III (10), was dialyzed against 100 mM sodium phosphate buffer, pH 7.5, and concentrated 10-fold by ultrafiltration. A typical 50-ml culture yielded 4 mg of protein with a specific activity of 6 × 10^6 dpm/mg.

CD Spectroscopy—A Jasco J-720 spectropolarimeter, interfaced to an Epson Equity 386/25 computer controlled by Jasco software, was used to analyze the α-helical content of mutant and wild-type (WT) apoLp-III and to monitor temperature or guanidine hydrochloride-induced denaturation (8). A modified Contin program by Provencher and Glöckner (20), which contains poly-L-glutamate as a helical reference standard, was used to estimate the α-helical content of the proteins.

Vesicle Clearance Assay—Binding of apoLp-III to dimyristoylphosphatidylcholine (DMPC) or dimyristoylphosphatidylglycerol (DMPG) vesicles was monitored by 90° light scattering. ApoLp-III has the ability to transform phospholipid vesicles to protein-lipid disc complexes. This process, which results in a significant reduction in size of the particles, can be monitored spectroscopically. To prepare multimellar vesicles of DMPC or DMPG (Avanti Polar Lipids Inc., Alabaster, AL), the phospholipid was dissolved in chloroform:methanol (3:1, v/v) and dried under a stream of N₂. Further dryness was achieved under vacuum for at least 4 h. The dried lipid sample was dispersed in pre-warmed buffer (10 mM Tris-HCl, pH 7.2; 150 mM NaCl; 0.5 mM EDTA) to a final lipid concentration of 10 mg/ml and vortexed for 1 min. From this solution, small unilamellar vesicles (~200 nm in diameter) were prepared by extrusion using 200-nm filters (21). A Perkin-Elmer spectrophotometer (model LS 50B) was used to monitor phospholipid vesicle clearance as a result of association with exchangeable apolipoproteins (22). Excitation and emission wavelengths were set at 600 nm with a slit width of 3 nm. The temperature of the cuvette holder was maintained at 23.9 °C (DMPC) or 23 °C (DMPG), and all solutions were preincubated at these temperatures. Lipid vesicles (250 μg) were added to 1 ml of buffer and equilibrated for 10 min in the cuvette holder. Specified apoLp-III (250 μg of protein for DMPC assays and 10 μg for DMPG assays) were then added, mixed for 10 s, and the change in light scattering monitored as a function of time.

Lipoprotein Binding Assay—Apolipoprotein binding to spherical lipoproteins was investigated using the assay system described by Liu et al. (23). Phospholipase C (PL-C)-mediated hydrolysis of the phosphatidylcholine component of human low density lipoprotein (LDL) results in accumulation of diacylglycerol in the monolayer, which leads to lipoprotein particle aggregation and development of sample turbidity (24). Although PL-C-mediated LDL aggregation is irreversible, it can be prevented by inclusion of amphipathic exchangeable apolipoproteins in the incubation. In the present study, LDL (50 μg of protein, isolated by sequential density ultracentrifugation (25)) was incubated with 0.16 units of Bacillus cereus PL-C (Sigma) (1 unit liberates 1 μmol of water-soluble organic phosphorus from egg yolk t-α-phosphatidylcholine per min at pH 7.3 at 37 °C) in the presence or absence of 40 μg of apoLp-III in buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 2 mM CaCl₂) at 37 °C. At indicated time points sample absorbance at 340 nm was determined.

Competition Binding Assay—A variation of the assay described above was used to compare the abilities of WT and mutant apoLp-III to initiate a stable binding interaction with PL-C-treated LDL. LDL (250 μg of protein) was treated with 0.88 units of PL-C in the presence of 3H-WT recombinant apoLp-III (250 μg of total culture medium protein) and indicated amounts of unlabeled competitor (WT or mutant) apoLp-III for 40 min at 37 °C. The reaction was stopped by the addition of KBr (2.8 x final concentration) and EDTA (3.5 mM final concentration) (26). The LDL fraction was re-isolated by KBr density gradient ultracentrifugation and 3H-WT apoLp-III bound to LDL determined by liquid scintillation spectrometry. Protein concentration was determined by the bicinchoninic acid protein assay (Pierce) and the specific activity expressed as dpm/mg protein.

RESULTS

Biophysical Properties—Loop regions that connect helices 1 and 2 and helices 3 and 4 in the globular α-helix bundle conformation of L. migratoria apoLp-III span residues 32–37 (loop A) and residues 88–96 (loop C). These loops possess hydrophobic character and have been suggested to interact with surface-localized diacylglycerol in lipoproteins to initiate stable binding (7). To test this hypothesis, specific leucine residues in the loops were converted to arginine, L32R, L34R, or L95R. A fourth mutant, where all the three Leu residues were mutated to Arg (L32R/L34R/L95R, referred to as the triple mutant), was also designed. The precise location of the mutations are depicted in Fig. 1. Before conducting functional analysis of the mutant apolipoproteins, their structural properties were char-

The numbering of apoLp-III starts with the first Arg residue of apoLp-IIIs. apoLp-IIIs is the mature protein resulting from signal peptide cleavage of the preproteins. A slow removal of the first two residues (Arg-Pro) is known to occur after secretion into the hemolymph (49, 50). All recombinant proteins used in the present study bear the two initial residues.
Biophysical properties of WT and mutant apoLp-IIIs

$T_m$ is the midpoint of temperature-induced denaturation (by ellipticity at 221 nm); [GdnHCl]$^{1/2}$ is the molar concentration of guanidine hydrochloride required to give a 50% decrease in ellipticity at 221 nm; $\Delta G_D^{H_2O}$, the free energy of unfolding in the absence of guanidine hydrochloride, was determined according to Pace (52) using the relationship $\Delta G_D = \Delta G_D^{H_2O} - m[GdnHCl]$, where $m$ is a constant that is proportional to the increase in degree of exposure of the protein upon denaturation.

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### Table I

| ApoLp-III      | $T_m$ | [GdnHCl]$^{1/2}$ | $\Delta G_D^{H_2O}$ |
|----------------|-------|------------------|---------------------|
| WT             | 55.0  | 0.61             | 2.68                |
| L32R           | 49.4  | 0.44             | 1.56                |
| L34R           | 49.6  | 0.43             | 1.51                |
| L95R           | 54.5  | 0.60             | 2.67                |
| L32R/L34R/L95R | 45.6  | 0.33             | 1.77                |
| M. sextaa      | 52    | 0.36             | 1.29                |

*a* For comparison, the data of *M. sexta* apoLp-III are shown (27).

characterized. CD spectroscopy revealed that each of the mutants possess a high $\alpha$-helical content (approximately 75%, results not shown). In temperature-induced denaturation studies monitored by CD, it was observed that WT apoLp-III showed an increase in content of random coil at 40 °C with a maximum reached at 70 °C. Upon cooling, the protein regains its helical structure indicating the process is reversible. Plots of ellipticity as a function of temperature revealed a 55 °C midpoint of temperature-induced denaturation. A similar reversible denaturation was observed for the mutant apoLp-IIIs, although lower denaturation midpoints were found for L32R, L34R, and the triple mutant (Table I). Introduction of Arg in positions 32 and 34 resulted in a slight decrease in the midpoint of GdnHCl-induced denaturation and corresponding $\Delta G_D^{H_2O}$ values, whereas substitution of Leu-95 by Arg did not result in any significant differences when compared with recombinant WT apoLp-III. Although variations in midpoints of temperature and GdnHCl denaturation occurred in the mutants, the values observed were in the range of other exchangeable apolipoproteins, i.e. the homologous *M. sexta* apoLp-III (see Table I) and human apoA-II ($\Delta G_D^{H_2O} = 1.0$ kcal/mol (28)). Thus, we conclude that the mutations introduced did not cause major changes in secondary structure content or stability.

**Phospholipid Vesicle Clearance**—When exchangeable apolipoproteins are mixed with unilamellar phospholipid vesicles at their transition temperature, discoidal protein-lipid complexes are formed (29). This process can be monitored by light scattering spectroscopy since the turbid vesicle solution (~200 nm in diameter) is clarified upon transformation to discoidal particles (~14 nm in diameter (8)). Two different phospholipid vesicles were examined as follows: negatively charged DMPG and zwitterionic DMPC. As shown in Fig. 2A, WT apoLp-III causes clearance of DMPG vesicle suspensions, whereas in the absence of apolipoproteins DMPG vesicles remain turbid. All single mutant apoLp-IIIs were able to cause DMPG vesicle clearance at 2–6-fold higher rates than WT apoLp-III, which required 95 s to achieve 50% of maximal clearance ($t_{1/2}$, see Table II). The triple mutant was 19-fold more efficient than WT apoLp-III in its ability to cause clearance ($t_{1/2} = 5$ s). Also, L32R and L34R apoLp-IIIs, with mutations in loop A, showed a more rapid interaction with DMPG vesicles than L95R apoLp-III (in loop C). To characterize further the effect of charge-charge interactions between phospholipid vesicles and the different mutants, clearance assays with zwitterionic DMPC vesicles were carried out. WT apoLp-III-induced clearance of DMPC vesicle turbidity is a slower process compared with DMPG, as judged by its higher $t_{1/2}$ (455 s), and the higher protein:lipid ratio (Fig. 2B). A stronger interaction of apolipoprotein for

![Image](image_url)

**Fig. 2.** Phospholipid vesicle clearance. DMPG (A) or DMPC (B) and apoLp-III were mixed using a phospholipid:protein ratio of 25:1 (DMPG) or 1:1 (DMPC). Vesicle clearance as a function of time was followed at the phospholipid transition temperature by 90° light scattering using a fluorescence spectrophotometer (excitation and emission wavelengths set at 600 nm).
TABLE II

Initiation of Lipoprotein Binding of ApoLp-III

| ApoLp-III | DMPC t_{1/2} | DMPG t_{1/2} |
|-----------|---------------|---------------|
| WT        | 95            | 455           |
| L32R      | 16            | 248           |
| L34R      | 17            | 32            |
| L95R      | 47            | 700           |
| Triple mutant | 5             | 130           |

DMPG compared with DMPC has also been observed for human apoA-I (29). The clearance rates varied with each mutant, with L34R and the triple mutant displaying the most effective clearance activity. L32R and L95R apoLp-IIIIs showed rates of clearance similar to WT apoLp-III.

Protection against PL-C-induced LDL Aggregation—The ability of WT and mutant apoLp-IIIs to bind to hydrophobic defects on a spherical lipoprotein surface was examined (Fig. 3). Treatment of isolated human LDL with PL-C creates surface-localized diacylglycerol, which destabilizes the LDL particle. This unstable, modified LDL tends to aggregate, resulting in sample turbidity which can be followed turbidimetrically. Inclusion of WT apoLp-III in the incubation prevents aggregation due to its ability to bind to the modified LDL surface (23). In the present study, WT and mutant apoLp-IIIs (L32R, L34R, and L95R) conferred complete protection against PL-C-induced LDL aggregation. The triple mutant, however, lost some of its ability to protect after extended incubation times. Control incubations of LDL without PL-C demonstrated no change in absorbance, indicating that LDL alone was stable under the conditions employed. We conclude that each of the four mutant apoLp-IIIs are capable of binding to lipolyzed LDL.

Competition Binding Assay—In order to compare the relative ability of WT and mutant apoLp-IIIs to initiate lipid binding, a competition binding assay was employed. In this case, PL-C treated LDL was incubated simultaneously with tritiated WT apoLp-III and a given unlabeled apoLp-III. These two “species” of apoLp-III then compete for binding sites created on the surface of LDL. The reaction was stopped after 40 min, a time point where WT apoLp-III fully protects LDL from aggregation. In experiments comparing the effect of increasing amounts of unlabeled WT competitor, a concentration-dependent reduction of LDL bound 3H-apoLp-III was observed (Fig. 4). Significant reductions in the relative amount of 3H-apoLp-III bound to LDL were observed using 50–400 μg of unlabeled recombinant WT apoLp-III. Thus, 250 μg of unlabeled competitor apolipoprotein was used in subsequent assays comparing WT and mutant apoLp-IIIs.

Inclusion of 250 μg of unlabeled WT apoLp-III caused reduction of LDL bound 3H-WT apoLp-III from 100 to 16%. However, equivalent amounts of L32R, L34R, or L95R apoLp-IIIIs were less effective competitors (Fig. 5). With these proteins LDL-associated radioactivity was reduced to 41 ± 7% (L32R), 43 ± 6% (L34R), and 53 ± 5% (L95R) under the conditions employed. These values are significantly different (p < 0.01) from that observed for WT apoLp-III, with little difference among the single mutants. The triple mutant showed a strongly decreased ability to compete with 3H-WT apoLp-III for binding sites created by PL-C, suggesting a cumulative effect of the mutations on its ability to initiate lipoprotein binding.

DISCUSSION

When the x-ray structure of L. migratoria apoLp-III was solved (7), one of the questions that arose related to the mode of interaction of this globular α-helix bundle protein with lipoproteins. It was postulated that neutral lipid accumulation on the surface of lipoproteins triggers binding of apoLp-III via confor-
Initiation of Lipoprotein Binding of ApoLp-III

The hydrophobicity of the loops of the single mutants, replacing candidate Leu residues with Arg, thereby dramati-
cered the first step in lipoprotein binding (18), followed by con-
formational opening of the helix bundle and spreading out on
the protein to compete with WT apoLp-III for binding to hy-
phobic sensor residues in lipoprotein binding by
such hydrophobic residues are likely involved in this role, their predomi-
national opening of the bundle (7). Such an opening (helices 1,
2, and 5 moving away from helices 3 and 4) was proposed to
involve putative hinge domains (loops B and D in Fig. 1). In this
model, apoLp-III exposes its interior to interact with a lipid
surface but can re-adopt the closed conformation following re-
lease from the lipoprotein surface. A similar mechanism has
been proposed for the N-terminal domain of human apoE
where lipid binding is essential for LDL receptor recognition (1,
30). This open conformation model has been supported by a
series of experiments reported over the last 5 years (8, 31–34).
Direct experimental evidence came from engineering a disul-
fide bridge in apoLp-III, locking the helix bundle in the lipid-
free closed conformation that resulted in abolition of lipopro-
tein binding (33). This leads to the intriguing question as to
how apoLp-III initiates lipoprotein binding. Whereas hydro-
phobic residues are likely involved in this role, their predomi-
nant localization within the interior of the helix bundle would
imply that partial unfolding of the protein has to occur. How-
ever, loops A and C, located at one end of the protein (29HETL-
GLPTPD 38 and 87SIHDAATSILN 386), were noted to contain hy-
drophobic amino acids. 3 Alignment of apoLp-III from several
insect species suggested the presence of conserved Leu residues
at or around positions 34 and 95 (17). These Leu residues were
suggested to function in recognition of potential binding sites
on the lipoprotein surface. This initial contact may be consid-
ered the first step in lipoprotein binding (18), followed by con-
formational opening of the helix bundle and spreading out on
the lipid surface. In this study, we have investigated the role of
such hydrophobic sensor residues in lipoprotein binding by
replacing candidate Leu residues with Arg, thereby dramati-
cally altering the hydrophobic character of the loop segment in
question. The hydrophobicity of the loops of the single mutants,
according to the normalized Eisenberg consensus (35), de-
creased from 0.70 to −2.89 (loop A) and from 1.19 to −2.40 (loop
C). On the other hand, loops B (67HQG 49) and D
(125SGEAWAPV 133) located at the opposite end of the helix
bundle (Fig. 1), are postulated to function as hinge domains,
about which the helix bundle opens. Unlike the Leu residues in
loops A and C, the two hydrophobic residues present in loop D
(Trp-130 and Val-133) are not conserved across species (17). Loop
B lacks hydrophobic residues. Thus, based on these consid-
ereations, we focused on Leu residues located in loops A and C.

The secondary structure content, denaturation properties,
and $\Delta G_D^{1 H_2 O}$ values of the mutants were similar to that of WT
protein and in the range observed for other exchangeable apo-
lipoproteins ($\Delta G_D^{1 H_2 O}$, $M. sexta$ apoLp-III: 1.68 kcal/mol (27);
apoA-I: 4.2 kcal/mol (36); apoA-II: 1.0 kcal/mol (28); and apoC-
II: 2.8 kcal/mol (37)). The midpoint of temperature-induced denaturation of $L. migratoria$ apoLp-III is similar to that of $M.$
sexta apoLp-III (55 and 52 °C, respectively). As described ear-
lier for $M. sexta$ apoLp-III (27), temperature-induced denaturation
of locust WT and mutant apoLp-III is completely revers-
ible. Of the three Leu residues in question, Leu-95 is the most
solvent-accessible residue (88%), 3 and its replacement by Arg
did not result in any change in the measured biophysical prop-
erties of apoLp-III. The observed small differences in the bi-
ophysical properties are likely due to local effects in the region
surrounding the mutations. Therefore, changes in the function
of the protein are likely a result of these local changes.

DMPC vesicle clearance data showed that substitution of
Leu for Arg in either loop A or C enhanced clearance rates. The
triple mutant displayed the highest rate, followed by both L32R
and L34R apoLp-IIIIs. L95R apoLp-III also displayed an in-
creased clearance rate, although the level of enhancement was
less than that observed with “loop A” mutant apolipoproteins.
Apparently, serial introduction of Arg results in a cumulative
increase in the ability to transform vesicles into discoidal com-
plexes. Therefore, the higher DMPC clearance rates observed
with mutant apoLp-IIIIs are likely a result of charge effects that
facilitate electrostatic attraction of apoLp-III to the negatively
charged DMPC vesicle surface. In addition, clearance studies
using zwitterionic DMPC vesicles showed that L34R apoLp-III
and the triple mutant interacted more rapidly with DMPC than
WT apoLp-III. Taken together, these results support the view
that it is the end of the apoLp-III bearing loops A and C that
initiates contact with these phospholipid surfaces.

Formation of a stable apoLp-III-lipoprotein interaction is
thought to result from replacement of helix-helix contacts in
the bundle conformation (Fig. 1) by helix-lipid interactions at
the lipoprotein surface through conformational opening (7).
Since the Leu residues replaced in the present study are local-
ized at one end of the molecule, their substitution by Arg is not
expected to result in abolition of lipoprotein binding. Indeed,
single mutants and the triple mutant (bearing three additional
positive charges in the loops) were able to bind to lipolyzed
LDL, although the triple mutant demonstrated a slightly de-
creased ability to protect over an extended period of time (Fig.
3). Since this turbidimetric binding assay is qualitative, we
developed a competition assay to quantify the interaction of
exchangeable apolipoproteins with PL-C-treated LDL. LDL
was co-incubated with 3H- and unlabeled apoLp-III (WT or
mutant), which compete for binding sites created on the surface
of LDL by the action of PL-C. This assay showed that the three
single Arg mutants were poor competitors compared with un-
labelled WT protein. Under the conditions employed, the ab-
ility of the single mutants to compete with WT apoLp-III for binding
sites on lipolyzed LDL was reduced 3-fold, with the triple
mutant eliciting a 5-fold reduction. These data indicate that
replacing Leu with Arg has a profound effect on the ability of
the protein to compete with WT apoLp-III for binding to hy-

3 Based on DSSP (51) analysis of the x-ray coordinates on deposit in
the Brookhaven Protein Structure Data Bank (code 1AEP).
diphospholipid vesicles. In the presence of hydrophobic surface defects on spherical lipoproteins, the initial encounter between apolipoprotein and vesicle is followed by penetration of the apolipoprotein into the bilayer, disrupting it to form disc-like particles. In these complexes apolipoprotein helical segments circumscribe the disc perimeter, covering otherwise exposed acyl chains (30, 31, 44). Unlike its interaction with DMPC or DMPC bilayer vesicles, apoLp-III does not form a stable binding interaction with the zwitterionic monolayer surface of LDL. Binding takes place in vitro, however, when hydrophobic binding sites (diacylglycerol) are created on the particle surface by the action of PLC. Importantly, diacylglycerol is the physiologically relevant substrate for apoLp-III, and this lipid is known to exist in the surface monolayer of low density lipoprotein (4, 45–48). Further study is ongoing to assess whether the present conclusions are applicable to helix bundle apolipoproteins in general.

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