Fluorescence Studies of Exchangeable Apolipoprotein-Lipid Interactions

SUPERFICIAL ASSOCIATION OF APOLIPOPHORIN III WITH LIPOPROTEIN SURFACES*

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Daisy Sahoo‡, Vasanthy Narayanaswami‡, Cyril M. Kay§, and Robert O. Ryan‡¶
From the §Lipid and the ¶Lipoprotein Research Group, Protein Engineering Network of Centres of Excellence, Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G 2S2, Canada

Apolipophorin III (apoLp-III) from the Sphinx moth, Manduca sexta, is an 18-kDa exchangeable apolipoprotein that reversibly associates with lipoprotein particles. In the absence of lipid, apoLp-III exists as an elongated bundle of five amphipathic α-helices. Upon lipid association, the protein is postulated to undergo a major conformational change, wherein the bundle opens around hinge loop regions, resulting in exposure of its hydrophobic interior. Fluorescence quenching techniques have been employed to study apoLp-III helix topology and spatial arrangement in phospholipid disc complexes and intact lipoprotein particles. Intrinsic fluorescence of the single tyrosine in apoLp-III was exploited to monitor the location of helix 5 in model disc complexes. To investigate other regions of the protein, site-directed mutagenesis was performed to introduce cysteine residues, replacing Asn-40 (helix 2, N40C) or Leu-90 (helix 3, L90C), thereby providing two mutant apoLp-III, each with a single site for covalent attachment of the extrinsic fluorescent probe, N-(1-pyrene) maleimide. In the lipid-free state, pyrene-N40C- and pyrene-L90C-apoLp-III were highly accessible to the negatively charged aqueous quencher KI, yielding $K_v$ values of 27.1 and 19.8 m$^{-1}$, respectively. Upon binding to the surface of a spherical lipoprotein particle, $K_v$ values for KI decreased by about 90% for both pyrene-labeled apoLp-III, indicating a significant change in the local microenvironment of the fluorophores. A lesser decrease in $K_v$ was observed when the pyrene-labeled apoLp-III were bound to phospholipid disc complexes. When spin-labeled fatty acids 5-doxystearic acid and 12-doxystearic acid were used as lipophilic quenchers, tyrosine and pyrene fluorescence were more effectively quenched by 5-doxystearic acid in both phospholipid bilayer disc complexes and spherical lipoprotein particles. These data provide insight into the spatial topology of apoLp-III α-helices in phospholipid disc complexes and support the concept that interaction with spherical lipoprotein particles results in superficial contact of apoLp-III helical segments with the monolayer surface, providing a basis for its reversible binding ability.

Plasma lipoprotein metabolism is regulated to a large extent by reversible binding of exchangeable apolipoproteins (1, 2). These amphipathic α-helical proteins have the ability to exist in both water-soluble and lipid-bound forms, a property which is the basis of their physiological role in lipoprotein metabolism. Apolipophorin III (apoLp-III) from the Sphinx moth, Manduca sexta, is a 166-amino acid member of the exchangeable apolipoprotein family that has been well characterized (3). Structural information is available for the lipid-free form of M. sexta apoLp-III (4), as well as Locusta migratoria apoLp-III (5). Both proteins exist as globular up-and-down amphipathic α-helix bundles, with their polar and charged residues facing the aqueous environment and their hydrophobic residues oriented toward the protein interior. Several lines of evidence support the view that lipid interaction of apoLp-III requires opening of the bundle, presumably around the hinge loop regions located between helices 2 and 3 and between helices 4 and 5 (5, 6). This conformational change would lead to exposure of the hydrophobic interior of the protein, facilitating lipid association. In this model, helix-helix interactions that maintain the structure of the bundle conformation are replaced by helix-lipid interactions. Chemical cross-linking and electron microscopy studies (8) and Fourier transform infrared spectroscopy (9) of apoLp-III in phospholipid bilayer discs indicate that the protein adopts an open state, oriented peripherally, surrounding the bilayer disc with its helices aligned perpendicular to the phospholipid fatty acyl chains. Although we have a good global view of how apoLp-III interacts with various lipid systems, key issues remain unresolved, including the molecular basis of conformational opening and the structure of the lipid-bound form.

To further understand the lipid-bound conformation of apoLp-III, we employed fluorescence techniques to study the accessibility to quenchers of the single tyrosine, as well as extrinsic fluorophores covalently bound to cysteine residues introduced at specified locations by site-directed mutagenesis. Although fluorescence from Tyr-145 (located in helix 5) is negligible in the lipid-free state (10, 11), a significant enhancement was observed upon lipid binding (8), which facilitated quenching studies. By comparison of the relative effectiveness of different lipid-based quenchers, information about the spatial alignment of apoLp-III α-helices complexed with phospholipids and spherical lipoprotein surfaces was obtained.

EXPERIMENTAL PROCEDURES

Materials—2,2′-dithiodipyrdine (Aldrich™), 5-doxystearic acid, and 12-doxystearic acid spin labels were obtained from Aldrich. N-(1-...
Pyrene-maleimide was purchased from Molecular Probes, Inc. (Eugene, OR). Dimyristoylphosphatidylcholine, phospholipase C from Bacillus cereus, and trifluoroethanol were purchased from Sigma, Sepharose CL-6B column matrix from Pharmacia Biotech Inc., and pET expression vector from Novagen (Madison, WI). All other chemicals and solvents were of analytical grade.

Site-directed Mutagenesis and Bacterial Expression—Site-directed mutagenesis of apoLp-III was performed as described earlier, using mismatched oligonucleotide primers (6). In one mutation, Asn-40 at the N terminus of helix 2 (close to the polar/nonpolar interface) was replaced by Cys (N40C). In another experiment, Leu-90 at the C terminus of helix 3 (in the center of the nonpolar face of the amphipathic helix) was replaced by Cys (L90C) (4). The genes encoding full-length apoLp-III bearing the mutations were cloned into the pET vector and transformed into E. coli BL21 cells as described by Ryan et al. (13).

Purification and Labeling of Recombinant apoLp-III—Wild-type, N40C-, and L90C-apoLp-III were purified by reversed phase high performance liquid chromatography as described previously (6). Electrospray mass spectrometry yielded a mass of 18,370 ± 1.5 and 18,371 ± 1.5 Da for N40C-apoLp-III and L90C-apoLp-III, respectively, as compared with 18,381 Da for wild-type apoLp-III (VG quattro electrospray mass spectrometer, Fisons Instruments, Manchester, UK). When the cysteine-containing mutant apoLp-III was labeled with 2,2′-dithiodipipyridine, a molecular mass increment of 108 daltons was observed. To label the cysteine mutants with N-(1-pyrene)maleimide, the probe was solubilized in dimethyl sulfoxide and incubated in a 2:1 molar ratio with either N40C- or L90C-apoLp-III (50 mM sodium phosphate, pH 7.0) for 2.5 h at 37 °C in the dark. Unbound pyrene was separated from labeled protein by gel filtration chromatography. The protein was analyzed by SDS-polyacrylamide gel electrophoresis to verify labeling and visualized under UV light.

Structural and Functional Characterization of Pyrene-labeled N40C- and L90C-apoLp-III—Circular dichroism spectra of pyrene-N40C-apoLp-III and L90C-apoLp-III (50 mM sodium phosphate, pH 7.0) were collected on a Jasco J-720 spectropolarimeter at 25 °C as described previously (13). The functional properties of pyrene-N40C- and pyrene-L90C-apoLp-III were characterized in a lipoprotein binding assay as described elsewhere (6).

Preparation of apoLp-III-DMPC Complexes—Wild-type apoLp-III, pyrene-N40C-apoLp-III, or pyrene-L90C-apoLp-III were complexed with DMPC as described by Weintzek et al. (8). The final lipid:protein molar ratio was 150:1.

Preparation of Lipoprotein-associated apoLp-III—Lipoprotein-associated wild-type, pyrene-N40C-, or pyrene-L90C-apoLp-III was prepared according to Liu et al. (14). Wild-type, pyrene-N40C-, or pyrene-L90C-apoLp-III (1.5 mg) was incubated with human low density lipoprotein (LDL) (2 mg protein) in the presence of 6 units of phospholipase C (from B. cereus) for 30 min at 37 °C. Unbound protein was separated by Sepharose CL-6B gel filtration chromatography.

Analytical procedures—Fluorescence measurements were performed on a Perkin-Elmer MFP-44B spectrophotometer with the temperature maintained at 20 °C in a thermostatted cell holder. A bandwidth of 4 nm was used for both excitation and emission monochromators. Excitation and emission spectra of pyrene-labeled apoLp-III was recorded by setting the emission and excitation wavelengths at 375 and 345 nm, respectively. Tyrosine fluorescence in wild-type apoLp-III (which lacks tryptophan) was measured at excitation and emission wavelengths of 277 and 300 nm, respectively. To measure the effect of aqueous quenchers, lipid-free pyrene-apoLp-III and DMPC-bound or LDL-bound pyrene-N40C- and pyrene-L90C-apoLp-III were treated with increasing concentrations of potassium iodide in 50 mM sodium phosphate, pH 7.5. KI solutions contained 1 mM sodium thiocyanate to suppress free iodine formation. Similarly, for depth-dependent quenching studies, aliquots of 5-doxylstearic acid or 12-doxylstearic acid (5-DSA and 12-DSA, respectively (1.3 mM stock in ethanol)) were added to lipid-bound samples (final concentration of ethanol, ≤1% by volume), and fluorescence intensities were measured following equilibration for 5 min. The effective quenching constants were determined according to the Stern-Volmer equation, $F/F_0 = 1 + K_{sv} [Q]$, where $F_0$ and $F$ are the fluorescence intensities in the absence and presence of quencher, respectively, and $Q$ is the concentration of quencher. The slope of the plot of $F/F_0$ versus $[Q]$ is the effective Stern-Volmer quenching constant, $K_{sv}$ (15). When using spin-labeled fatty acids as quenchers, the classic Stern-Volmer equation for free diffusion no longer applies because the quencher is not uniformly distributed over the corresponding space coordinate (16, 17). Therefore, of purposes of relative comparison within spin-labeled fatty acids, we have calculated an apparent quenching constant ($K_{qv}$) in place of $K_{sv}$.

Analytical procedures—SDS-polyacrylamide gel electrophoresis was performed on 12% acrylamide slab gels run at 30 mA constant current and stained with Coomassie Brilliant Blue R-250. Protein concentrations were determined by the bichinonic acid assay (Pierce), and phosphatidylcholine was determined using an enzyme-based colorimetric assay (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

RESULTS

Structural and Functional Characterization of Alkylated N40C- and L90C-apoLp-III—Modification of N40C- and L90C-apoLp-III by covalent attachment of pyrene maleimide resulted in labeling of the single engineered cysteine residue. The presence of a single attachment site on either protein and the availability of the proteins for alkylation were examined by two independent methods. The first method used 2,2′-dithiodipipyridine alkylation, which under native conditions resulted in covalent linkage of the thiopyryl moiety to the cysteine, yielding a product with a molecular mass increment of 108 Da. The second method employed pyrene maleimide, which upon covalent linkage to the free sulhydryl yielded a product with a molecular mass increment of 297 Da (from 18,371 to 18,668 Da). These data indicate that there is only one cysteine present in both recombinant proteins and that, under the conditions studied, they are not involved in intermolecular disulfide bond formation. N-1-Pyrene/maleimide has the unique property of becoming fluorescent only after covalent attachment to a sulphydryl group. Following SDS-polyacrylamide gel electrophoresis, N40C-apoLp-III and pyrene-L90C-apoLp-III were fluorescent when viewed under a UV illuminator. Fluorescence excitation and emission spectra of pyrene-N40C- and L90C-apoLp-III are shown in Fig. 1. Typical fluorescence properties of pyrene, such as excitation at 335 and 345 nm and emission at 375 and 396 nm, with a high quantum yield, are evident, confirming labeling of the cysteine residue on the proteins. Circular dichroism spectra revealed that the secondary structure content of pyrene-N40C- and pyrene-L90C-apoLp-III (60% α-helix) is very similar to that of wild-type apoLp-III (10).
Fluorescence properties of labeled apoLp-IIIIs were used to investigate the effect of lipid interaction on specific apoLp-III helical segments. A novel method was employed to induce a stable binding interaction between labeled apoLp-IIIIs and spherical lipoprotein particles. This generally applicable method takes advantage of the ability of exchangeable amphipathic apolipoproteins to stabilize human LDL under conditions in which neutral glycerolipid is exposed on the particle surface (14). Upon treatment of LDL with phospholipase C, the polar head groups are cleaved from phosphatidyicholine moieties, resulting in the exposure of diacylglycerol on the monolayer surface (18). Creation of this hydrophobic surface induces aggregation of LDL particles, as seen by increased sample turbidity as a function of time (Fig. 2). However, in the presence of wild-type apoLp-III, aggregation is prevented due to a stable binding interaction with the modified LDL. The observation that pyrene-N40C- and pyrene-L90C-apoLp-III were able to prevent phospholipase C-induced LDL aggregation demonstrates that covalent attachment of the extrinsic fluorophore does not disrupt their lipoprotein binding ability and provides a useful system to study apolipoprotein interaction with the surface of a spherical lipoprotein.

**Potassium Iodide Quenching**—The aqueous quencher, KI, was used to probe the accessibility and alteration in the local microenvironment of pyrene-labeled apoLp-IIIIs in lipid-free and lipid-bound states (19). Table I shows the effective Stern-Volmer quenching constants ($K_{sv}$) obtained for pyrene-N40C- and pyrene-L90C-apoLp-III. Iodide, which quenches by a dynamic, collisional mechanism, was an efficient quencher of pyrene-N40C- and pyrene-L90C-apoLp-III fluorescence in the absence of lipid. When pyrene-labeled N40C-apoLp-III was unfolded in 2 M guanidine HCl, the observed $K_{sv}$ (30.5 m$^{-1}$) was similar to that for the native protein, indicating that the pyrene moiety in native N40C-apoLp-III is highly accessible to KI. With pyrene-N40C-apoLp-III-DMPC complexes, the extent of quenching by iodide was similar to that in the lipid-free state. On the other hand, with pyrene-L90C-apoLp-III-DMPC complexes, $K_{sv}$ decreased by nearly 40% compared with the lipid-free state. This reduced quenching efficiency may be due to a change in the microenvironment of the fluorophore upon interaction with the disc particles. Alternatively, it may be that the hydrophobic pyrene molecule is buried in the interior of the phospholipid bilayer, thereby denying access to iodide. Iodide was less effective at quenching pyrene-N40C- or pyrene-L90C-apoLp-III fluorescence when bound to the surface of LDL particles. The observed significant reduction in $K_{sv}$ values indicates sequestration of the fluorophore in both labeled proteins away from the aqueous environment.

**Lipophilic Quenchers**—Spin-labeled fatty acids, with the doxyl moiety located at different positions along the hydrocarbon backbone of the fatty acyl chain, serve as useful quenchers that operate within a lipid environment (20). These quenchers may be used as “molecular rulers” to characterize the interaction of a fluorophore with a given lipid surface. 5-DSA and 12-DSA were incorporated into lipid particles and used to estimate the spatial disposition of Tyr-145 in wild-type apoLp-III and the pyrome moiety on N40C- and L90C-apoLp-III, respectively. Upon binding DMPC, the globular apoLp-III helix bundle undergoes a conformational opening, aligning its helical segments around the perimeter of the disc complexes (8, 9). Quenching of tyrosine and pyrene fluorescence upon binding of apoLp-III to DMPC bilayer discs is shown in Figs. 3 and 4, respectively. A plot of $F/F_{0}$ versus $[Q]$ yields a straight line, the slope of which is equivalent to the apparent quenching constant, $K_{app}$ (inset in Figs. 3 and 4). Table II shows that 5-DSA quenched tyrosine fluorescence to a significantly larger extent than 12-DSA. Similarly, in both pyrene-N40C-apoLp-III/DMPC and pyrene-L90C-apoLp-III-DMPC complexes, a greater extent of quenching was observed with 5-DSA.

In contrast to the location of apoLp-III in DMPC complexes, where it interacts with the phospholipid fatty acyl chains around the perimeter of the disc, apoLp-III likely binds to the surface monolayer of spherical lipoproteins with its helices between the head groups of phospholipids in the surface monolayer (21, 22). To evaluate whether apoLp-III localizes superficially or actually makes direct contact with the particle interior, the ability of 5-DSA versus 12-DSA to quench pyrene-apoLp-III fluorescence was examined (Fig. 5). This experimental approach could not be applied to wild-type apoLp-III, however, because its tyrosine fluorescence is masked by that of the apoB-100 component of LDL. With both LDL-associated pyrene-N40C-apoLp-III and pyrene-L90C-apoLp-III, 5-DSA quenched to a much greater extent than 12-DSA, suggesting that the fluorophore is located superficially, close to the water/lipid interface.

**DISCUSSION**

The helix-bundle molecular architecture is a common structural motif of exchangeable apolipoproteins in their lipid-free

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**Fig. 2. Effect of apolipoproteins on phospholipase C-induced turbidity of LDL.** Fifty µg of pyrene-N40C-apoLp-III (filled triangles), L90C-apoLp-III (open triangles) or wild-type apoLp-III (filled circles) were incubated with isolated human LDL (50 µg protein) in the presence of 160 milliunits phospholipase C in a final volume of 250 µl in a microtiter plate at 37 °C. A negative control that had LDL but no added apoLp-III (open squares) was included. The reaction was initiated by the addition of phospholipase C (open squares) and a positive control that had LDL but no added apoLp-III (filled squares) were included. The reaction was initiated by the addition of phospholipase C, and the turbidity was measured at 600 nm at the specified times. Values are the average of three independent measurements ± S.E.
state (4, 5, 23). Such an organization facilitates its existence as
a soluble protein in an aqueous medium, such as plasma, and
permits binding to lipid surfaces through a conformational
change. To obtain information about lipid-associated conforma-
tions of apoLp-III, the fluorescence properties of its unique
tyrosine residue (located close to the center of the nonpolar face
at position 145 in helix 5) have been investigated (8, 10, 11, 24).
In these studies, Tyr-145 served as a reporter of the conforma-
tional status of helix 5 in different environments. However,
examining intrinsic tyrosine fluorescence has limited applica-
tions for the following reasons: 1) its quantum yield is low, 2) it
cannot be selectively evaluated in the presence of other pro-
teins, and 3) the focus is limited to helix 5. To circumvent these
problems and expand the versatility of the fluorescence ap-
proach to investigating structural aspects of lipid-associated
apoLp-III, we have introduced single cysteine residues into the
protein by site-directed mutagenesis at Asn-40 (helix 2) or
Leu-90 (helix 3). Following covalent attachment of pyrene to
the free sulfhydryl, fluorescence quenching was performed to
evaluate the orientation of apoLp-III helical segments in
DMPC disc complexes and the depth of penetration of the
helices into the monolayer surface of intact spherical lipopro-
tein particles. Advantages of this approach include the high
quantum yield of the pyrene fluorophore, the lack of interfer-
ence from other proteins that may be present in the system
under study, and the ability to introduce the reporter fluoror-
phore at specified locations in the protein.

Changes in accessibility to quenching by KI were measured to
monitor alterations in the local microenvironment of the
labeled cysteine residues. In the lipid-free globular form, high
quenching constants were obtained for both pyrene-N40C- and
pyrene-L90C-apoLp-III. This indicates that the pyrene moiety
is readily accessible to the negatively charged iodide. Exami-
nation of the structure of lipid-free apoLp-III (4) revealed sev-
eral lysine residues in helices 3 and 4 in close proximity to
N40C, as well as at positions i, i + 1 and i, i + 4 in helix 2.
Similarly, lysines were found in helix 4, as well as at the i, i +
2 position to L90C in helix 3. Thus, a positively charged micro-
environment in the vicinity of the fluorophore likely facilitates
iodide quenching. Upon binding to model disc complexes, a 40%
decrease in $K_{sv}$ was noted for pyrene-L90C-apoLp-III, yet little
or no change in $K_{sv}$ was observed for pyrene-N40C-apoLp-III
in these complexes. This result may be explained if helix 2 is
located closer to the quaternary amine of choline head groups
of the phospholipids, promoting iodide quenching (25). When
bound to the surface of spherical lipoprotein particles, $K_{sv}$ for
both pyrene-labeled apoLp-IIIIs decreased by about 90% com-
pared with the lipid-free state. A likely explanation for this
large decrease in $K_{sv}$ is burial of the hydrophobic pyrene moiety
in the lipoprotein monolayer, which denies access to the hydro-
phobic iodide. Taken together, these results document clear
differences in the microenvironment of the probe upon associa-
tion with phospholipid disc complexes versus lipoprotein par-
ticles, indicating that these represent distinct lipid surfaces for
binding of exchangeable apolipoproteins. Furthermore, apoli-
ipoprotein/lipoprotein complexes created using phospholipase
C-modified LDL provide a useful, structurally relevant alter-
native to phospholipid discs for studying the properties of lipid-
associated exchangeable apolipoproteins. Indeed, these parti-
cles are amenable to studies of the depth of penetration of
apolipoprotein helical segments into the lipoprotein surface.

**Use of Fatty Acid Spin Labels to Study Lipid-bound Protein
Conformations**—Protein-lipid interactions in model mem-
branes have been studied in detail using spin-labeled fatty
acids (26). Fatty acid spin labels quench via a collisional me-
chanism that is short-range in nature. Thus, they have to be
present in or near the sphere of action of the fluorophore to
quench effectively, with an optimal interaction distance of 4–6
Å (27). We have estimated the transverse disposition and depth
Lipid-bound Conformations of Apolipopophorin III

Table II
Apparent Stern-Volmer constants ($K_{\text{app}}$) for wild-type and pyrene-labeled apoLp-III using spin-labeled fatty acids

|                | 5-DSA               | 12-DSA              |
|----------------|---------------------|---------------------|
|                | $K_{\text{app}}$  | $K_{\text{app}}$  |
| DMPC-bound     | $0.21 \pm 0.04$     | $0.09 \pm 0.01$     |
| LDL-bound      | ND                  | ND                  |

$^a$ $K_{\text{app}}$ values were calculated as described under “Experimental Procedures”; values shown are the mean ± S.E. ($n = 3$).
$^b$ ND, not determined due to interference of fluorescence from apoB-100.

of penetration of apoLp-III helices in model disc complexes and lipoprotein particles, respectively, based on the parallax fluorescent quenching method originally proposed by Chattopadhyay and London (28). This method recommends the use of spin-labeled phospholipids instead of spin-labeled fatty acids due to the vertical fluctuations and relatively faster lateral mobility of the latter (29). However, our attempts to make discs containing spin-labeled phospholipids were unsuccessful as judged by electron microscopic characterization of the product particles. The choice to use spin-labeled fatty acids in our experiments was to our advantage because they can be added directly to the lipoprotein or disc system with bound apoLp-III. Quenching by spin-labeled fatty acids has been successfully used in several cases to measure the location of protein-bound fluorophores in membranes by comparison of the quenching efficiency of probes placed at various depths in the membrane (30–32). In 5-DSA, the doxyl moiety is located approximately 6 Å from the membrane/water interface, whereas in 12-DSA, it is located about 15 Å from the interface, deep in the membrane interior (20).

Fluorescence Quenching of apoLp-III-Phospholipid Disc Particles—Wild-type apoLp-III contains a single tyrosine residue, which is an intrinsic fluorophore. Previous studies have shown that helix 5, in isolation, cannot form complexes with phospholipid in vitro (11). However, quenching of its fluorescence by spin-labeled fatty acids when helix 5 is present in the intact protein indicates that despite its terminal location in the protein sequence, helix 5 contacts the lipid surface in a stable, oriented manner. Pyrene-labeling of two cysteine mutant apoLp-IIIIs allowed further quenching studies, yielding information on the disposition of helices 2 and 3, as well. In the lipid-free protein, NMR structural studies revealed that helix 5 interacts with helices 1 and 2, whereas there are no contacts between helices 1 and 2 themselves (4). In studies of the conformation of apoLp-III in phospholipid disc particles, cross-linking experiments indicate that about six molecules of apoLp-III align themselves around the circumference of the disc complexes (8). On the basis of Fourier transform infrared spectroscopy studies, it is concluded that the helices adopt a unique orientation, with their axes oriented perpendicular to the fatty acyl chains of the phospholipid bilayer (9). If conformational opening occurs as proposed, both ends of the molecule should contact the lipid surface. The quenching studies with pyrene-N40C- and pyrene-L90C-apoLp-III support this view. Furthermore, because 5-DSA displayed a more pronounced quenching efficiency than 12-DSA in the disc complexes, the helices containing Tyr-145 (helix 5), pyrene-N40C (helix 2), and pyrene-L90C (helix 3) likely align closer to the water/lipid interface. These data suggest that helix alignment in these complexes is not random but rather that the protein adopts a preferred orientation with respect to the bilayer. Further studies will be required to delineate the precise location of the different helices with respect to each other and to the phospholipid bilayer.

Superficial Association of apoLp-III with Lipoprotein Surfaces—All exchangeable apolipoproteins identified to date share a common amphipathic α-helix structural motif (33). These helices have well-defined polar and nonpolar faces that can interact with the aqueous and hydrophobic milieu, respectively, on a lipoprotein surface. This feature imparts to exchangeable apolipoproteins the structural adaptability to reversibly associate with circulating lipoproteins. We have examined the association of apoLp-III with lipoprotein surfaces by first allowing the pyrene-labeled protein to bind to the surface of LDL particles modified by treatment with phospholipase C, a situation that mimics the physiological setting wherein apoLp-III binds to lipoprotein particles in response to diacylglycerol enrichment (34). We have estimated the location of the fluorophore within the lipoprotein surface monolayer by comparing the quenching obtained with spin labels at two different depths. With pyrene-N40C-apoLp-III or pyrene-L90C-apoLp-III, the fluorophore was quenched 6–10-fold more with 5-DSA than with 12-DSA. Thus, it is estimated that the helices are located near the water/lipid interface with their axes perpendicular to the phospholipid monolayer. Based on the hinged loop opening, it may be envisaged that the hydrophobic interior of the protein is superficially embedded with its helical axes parallel to the lipoprotein surface in contact with the diacylglycerol-containing phospholipid monolayer (22). Because we are monitoring fluorescence at both ends of the protein (in its open conformation), this superficial binding is representative of
the entire protein, implying a “loose binding” of apoLP-III with the lipoprotein surface. The superficial mode of binding of apoLP-III to the lipoprotein surface is functionally relevant given the reversible nature of exchangeable apolipoprotein interactions. This would facilitate dissociation from lipid surfaces without a large input of energy and is consistent with displacement studies performed by Liu et al. (35) in which apoLP-III was readily displaced from the surface of low density lipophorin by human apoA-I. A similar mode of interaction has been proposed for the binding of class A amphipathic helices of apolipoproteins with the phospholipid monolayer, although a deeper penetration of the nonpolar face has been implied (36). In the case of apoLP-III, the binding interaction appears to be correlated with exposed diacylglycerol, which probably acts as a trigger for initiating association with the lipoprotein surface.

Compared with the lipid-free state, there is a lack of information regarding the lipid-bound conformations of exchangeable apolipoproteins. X-ray crystallography and NMR are limited by the large size and heterogeneity of lipid-protein complexes. Several groups have used alternative methods to gain insight into the lipid-bound conformations of apolipoproteins. Epitope mapping (37), Fourier transform infrared spectroscopy (38), computer modeling (39), and synthetic peptide fragment studies (7, 40) have been used to investigate this issue. All of the above studies employed protein systems for which the lipid-free structures remain unknown. On the other hand, we have focused on the topography of a full-length apolipoprotein, the structure of which in the lipid-free state is known (4). Using a combination of protein engineering, design, and fluorescence spectroscopy techniques, we have examined aspects of lipid-binding induced helix realignment and the spatial disposition of apoLP-III helical segments with respect to model disc complexes and a lipoprotein surface.

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