Effects of Phospholipid on the Structure of Human Apolipoprotein A-IV*

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We have used fluorescence and circular dichroism spectroscopy to investigate the effect of phospholipid on the structure and molecular stability of human apolipoprotein A-IV (apo-A-IV). Binding of apo-A-IV to egg phosphatidylcholine vesicles was rapid and did not cause release of encapsulated 6-carboxyfluorescein. Fluorimetric titration established that apo-A-IV bound to the vesicles with an association constant of $3.6 \times 10^6$ liters/mol and a binding maximum of 2 molecules per vesicle. Binding of apo-A-IV to the vesicle surface caused a progressive increase in $\alpha$ helicity from 43% at baseline to 83% at saturation; denaturation studies showed that the free energy of stabilization of binding was 6.31 kcal/mol. Fluorescence quenching studies revealed that binding of apo-A-IV to the vesicles was associated with a dramatic decrease in the fractional exposure of tyrosine to iodide, and a decrease in the efficiency of intramolecular tryptophan-tryptophan energy transfer. These findings suggest that the binding of apo-A-IV to the vesicle surface may involve a relaxation of the globular protein conformation in which the tyrosine containing $\alpha$-helical domains surrounding the tryptophan "unfold" and reorient their hydrophobic faces toward the phospholipid monolayer, with a consequent induction of additional $\alpha$-helical structure. However, our data also suggest that apo-A-IV does not penetrate deeply into the region of the phospholipid fatty acyl chains, but rather sits higher in the monolayer, intercalated between the charged phospholipid head groups. This characteristic may determine the labile interaction of apo-A-IV with high density lipoproteins.

Human apolipoprotein A-IV (apo-A-IV) is a plasma glycoprotein of molecular weight 46,000 (1–3) which is synthesized in the enterocytes of the small intestine (4–6). Although the specific function of apo-A-IV in human lipid metabolism has not yet been established, several lines of evidence suggest that apo-A-IV participates in the intravascular metabolism and remodeling of HDL. Apo-A-IV has been shown to activate lecithin-cholesterol acyltransferase, a key enzyme in the esterification of HDL cholesterol (7, 8). We have demonstrated that the action of lecithin-cholesterol acyltransferase changes the in vitro lipoprotein distribution of human apo-A-IV in a manner that parallels the formation and exchange of cholesterol esters (9). Apo-A-IV may be an activator for an "HDL conversion factor" which effects the in vitro interconversion of HDL subfractions (10). Recently, apo-A-IV has been shown to promote cellular cholesterol efflux (11) and thus may participate in the process of reverse cholesterol transport.

The biochemical and biophysical behavior of human apo-A-IV is quite distinctive from that of other human apolipoproteins. Although apo A-IV contains a high content of amphiphilic $\alpha$-helical structure (12), it is the most hydrophilic human apolipoprotein (13), and its affinity for lipid surfaces is very labile; consequently, it is readily displaced from the surface of native and model lipoproteins by other soluble apolipoproteins (14, 15). In this regard, we have proposed that the hydrophobic domains of apo-A-IV may be oriented toward the interior of the protein where they are unavailable for binding to lipid surfaces (16). Furthermore, apo-A-IV readily undergoes self-association in vitro and in vivo and forms dimers with an unusually high affinity (17). Perhaps because of these characteristics, apo-A-IV circulates primarily unassociated with serum lipoproteins (5, 18–20). The unusual properties of human apo-A-IV raise the question as to whether it is a "true" apolipoprotein, able to bind to the surface of lipoproteins in an energetically favorable manner, with a consequent stabilization of its ordered structure. The effect of lipid on the structure of human apolipoproteins has been extensively studied by a variety of physical methods (21–23); however, to date, such techniques have not been applied to the study of apo-A-IV/lipid recombinants. As knowledge regarding the interaction of apo-A-IV with lipid surfaces will be critical to the elucidation of the mechanisms by which it participates in intravascular lipoprotein metabolism, we have used fluorescence and circular dichroism spectroscopy to investigate the effect of phospholipid on the structure and molecular stability of human apo-A-IV.

EXPERIMENTAL PROCEDURES

Isolation of Apolipoprotein A-IV. Human apo A-IV was isolated from lipoprotein-depleted serum ($d > 1.25$ g/ml) by adsorption to a phospholipid-triglyceride emulsion followed by delipidation and anion exchange chromatography in 7.2 M urea (12). Immediately after chromatography, apolipoprotein solutions were desalted and concentrated to $1 \text{mg/ml}$ by diafiltration against 50 mM Tris, pH 7.4, 1 mM EDTA, 0.02% sodium azide in an Amicon 8050 cell with a YM-10 membrane. Protein solutions were sterilized by passage through a 0.22-$\mu$m filter and stored at 4°C. Protein concentration was measured using the BCA protein assay (24). Purity was established by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and by reduction and polyacrylamide gradient gel electrophoresis. Preparation of Phospholipid Vesicles—Undiluted egg yolk phosphatidylcholine vesicles (EPCV) were prepared by sonication. Ten-milligram aliquots of egg yolk 1-$\alpha$-phosphatidylcholine (Calbiochem)
in chloroform were placed in Pyrex glass tubes (10 x 60 cm), and the solvent was removed with a stream of dry nitrogen. The phospholipid was then hydrated with 1 mL of 50 mM Tris, pH 7.4, 1 mM EDTA, 0.02% sodium azide, and sonicated (Laboratory Supplies, Hicksville, NY) under nitrogen for 10 min at 24 °C at full power. The optically clear solutions were then pooled and centrifuged in Ultraclear (Beckman) tubes in a Ti 40.5 rotor at 40,000 rpm for 3.5 h at 25 °C. The supernatants containing unilamellar vesicles (25) were aspirated from the tubes and concentrated in an Amicon ultrafiltration cell with an XM-50 membrane. The phospholipid concentration of each preparation was determined by the method of Bartlett (20) and averaged 17.6 ± 1.5 mg/mL. Analysis of the EPCV by gel filtration chromatography on a calibrated 50-cm Sephacryl CL-4B column revealed a homogeneous population with an average Stokes radius of 1.06 ± 10° and an average Stokes radius of 9.5 nm.

EPCV containing the self-quenching fluorescent dye 6-carboxyfluorescein were prepared by including 200 mM 6-carboxyfluorescein in the buffer solution used to hydrate the egg yolk phosphatidylcholine (27). Following sonication, unincorporated dye was removed by gel filtration on a 25-cm column of Sephadex G-25 eluted with Tris buffer at a rate of 60 mL/h. Unilamellar vesicles were then isolated and concentrated as previously described. The phospholipid concentration of the resulting preparations was 1.75 mg/mL. When excited at 470 nm, 6-carboxyfluorescein containing EPCV demonstrated negligible fluorescence in Tris buffer; a bright emission at 515 nm appeared immediately upon disruption of the vesicles by addition of 10% sodium dodecyl sulfate.

Preparation of Apo-A-IV Phospholipid Micellar Complexes Apo-A-IV-phospholipid micellar complexes (MC) were made by cholate dialysis (28). Apo-A-IV was combined with egg yolk phosphatidylcholine and cholesterol in a molar ratio of 1:500:25. Aliquots of phospholipid and cholesterol dissolved in chloroform were added to glass tubes and dried under a stream of nitrogen. Apo-A-IV dissolved in 50 mM Tris, pH 7.5, 100 mM sodium chloride was added, and a sufficient quantity of 750 mM sodium chloride in this same buffer was added to yield a final cholate concentration of 60 mM in all instances. The fractional exposure of tryptophan and tyrosine was calculated as K, (apo-A-IV)/K, (N-acetyltryptophanamide or N-acetyltirosine). Fluorescence spectra were obtained at 0.5 nm intervals between 250 and 310 nm using calcite prism polarizers, the emission monochromator set at 370 nm, 8 nm monochromator slits, and a 0.5-s integration rate.

The efficiency of energy transfer between tyrosine and tryptophan was determined by analyzing the wavelength dependence of the relative tryptophan quantum yield (30). The ultraviolet absorption of the solutions was measured by using a Hewlett-Packard 8450A diode array spectrophotometer and corrected for buffer or phospholipid blanks. Fluorescence emission spectra were then monitored at 370 nm while the excitation wavelength was scanned from 270 to 295 nm. The data were then fitted to the expression:

$$\frac{[I(\lambda)/[A(\lambda)]/[I_{em}/A_{em}]} = [1 - e(\lambda)] + e$$

where λ is the fluorescence intensity at a given wavelength in arbitrary units, A(λ) is the absorbance of the solution at the same wavelength, e(λ) is the fractional absorption of tryptophan in apo-A-IV at a given wavelength (obtained from model solutions of N-acetyltryptophanamide or N-acetyltirosine), and e is the efficiency of energy transfer (16).

The affinity of apo-A-IV for phospholipid and the stoichiometry of binding to EPCV was determined by equilibrium fluorescence titration (31). Increasing concentrations of EPCV were added to solutions of apo A-IV at two different concentrations (C1 = 188 mM, C2 = 550 mM), and the areas under the emission spectra from 300 to 370 nm, corrected for dilution, were determined. The maximum fluorescence intensity for each titration was determined from the y-intercept of a plot of 1/fluorescence intensity versus [apo-A-IV]/[EPCV]. The fluorescence intensity at each phospholipid concentration was then normalized as $\Delta M_{max}$ (where $\Delta M$ is the increase in intensity following each addition of EPCV, and $\Delta M_{max}$ is the difference between the maximum and base-line fluorescence intensity) and plotted against the phospholipid/apo-A-IV ratio. Extrapolation of the initial linear portions of the curves, which correspond to the region of stoichiometric binding, yielded $M_1$ and $M_2$, the lipid/protein ratio at saturation. The association constant, $K_0$, and the binding maximum, $B_{max}$, were calculated using the formulas (31):

$$K_0 = \frac{[C_1/M_2 - C_1/M_1]/[C_2/M_1 - M_2]}{[C_1 - C_1/M_1]}$$

$$B_{max} = \frac{[C_1/M_1 - C_1/M_2]/[C_1 - C_1]}{[C_1 - C_1/}$$

Circular Dichroism Spectroscopy—The circular dichroism of solutions of apo-A-IV and apo-A-IV/phospholipid recombinants was measured using a Jasco J 500A Spectropolarimeter interfaced with an on-line data processor. The instrument was calibrated with 10-d- camphorsulfonic acid. Spectra were recorded from 190 to 260 nm at a scan speed of 600 nm/min, with a 5-nm bandwidth and a 2-s time constant. Each spectrum was the average of three scans; appropriate blanks were digitally subtracted. Mean residue ellipticity at 222 nm (θ222) and percent α-helicity were calculated as previously described (12). Titration studies were performed by monitoring θ222 following addition of increasing amounts of EPCV to solutions of apo-A-IV. Denaturation studies were performed by the sequential addition of aliquots of buffered 8 M guanidine hydrochloride to solutions of apo-A-IV and apo-A-IV/phospholipid recombinants.

**RESULTS**

Rate and Affinity of Binding to Phospholipid Vesicles—The rate of binding of apo-A-IV to EPCV was examined by monitoring time-dependent changes in fluorescence intensity and circular dichroism. Addition of EPCV to a 550 mM solution of apo-A-IV caused an immediate 1.5-fold increase in fluorescence intensity which was 90% of maximum at 1 min and reached a plateau by 15 min (Fig. 1). The increase in the mean residue ellipticity at 222 nm, θ222, which correlates with α-helicity, was more gradual and was 54% of maximum at 1 min and reached a plateau at 30 min. Binding of apo-A-IV to the vesicles did not alter their stability, as evidenced by the absence of release of encapsulated 6-carboxyfluorescein (Fig. 1, inset).

Fluorometric titration was utilized to determine the affinity of apo-A-IV for EPCV and the stoichiometry of binding. Addition of increasing concentrations of EPCV to solutions of apo-A-IV at two different concentrations caused a progressive increase in fluorescence intensity. The plots of the normalized increase in intensity versus the lipid/protein ratio...
The mean residue ellipticity at 222 nm, \( \kappa \), which reached a plateau of 29,667 degrees cm\(^{-1}\) dmol\(^{-1}\) (Fig. 3). These values correspond to an increase in calculated \( \alpha \)-helicity from 43% to 62%, although it depends on the lipid/apoprotein ratio. It is likely that the amount of \( \alpha \)-helical structure in such complexes decreases proportionately less, suggesting that the tyrosine residues were sequestered in a region shielded by a negative charge. In comparison, the tyrosine residues in apo-A-IV/MC displayed smaller decrements in fractional exposure to both iodide and acrylamide (Table I). However, the fractional exposure of tyrosine to acrylamide decreased proportionately less, suggesting that the tyrosine residues were inaccessible to this negatively charged quencher. The fractional exposure of tyrosine to acrylamide decreased proportionately less, suggesting that the tyrosine residues were sequestered in a region shielded by a negative charge. In comparison, the tyrosine residues in apo-A-IV/MC displayed smaller decrements in fractional exposure to both quenchers, particularly iodide. The fractional exposure of tryptophan in the apo-A-IV/MC to iodide was less than that for the EPCV.

We next examined the effect of lipid binding upon intramolecular tyrosine-tryptophan energy transfer in apo-A-IV. The ratio of fluorescence anisotropy at 305 nm to that at 270 nm gives a qualitative measure of the efficiency of energy transfer (32); values greater than 1.50 are indicative of increasing efficiency. \( A_{305}/A_{270} \) was 2.24 for apo-A-IV in Tris buffer and 1.92 for apo-A-IV in the presence of a saturating concentration of EPCV. Determination of the excitation wavelength dependence of the relative tryptophan quantum yield allowed quantitation of the decrease in energy transfer efficiency. For native apo-A-IV, the observed values for the relative tryptophan quantum yield were best fit by a curve calculated for an energy transfer efficiency of 0.52 (Fig. 4). The exposure and electronic interaction of tyrosine and tryptophan residues. Apo-A-IV in Tris buffer displayed a maximum tryptophan fluorescence emission at 333 nm. The addition of a saturating concentration of EPCV to apo-A-IV solutions caused a blue shift in the wavelength of maximum fluorescence emission to 330 nm. In comparison, the tryptophan emission of apo-A-IV complexed in MC was blue-shifted only 1 nm to 332 nm. Fluorescence quenching studies revealed that binding of apo-A-IV to EPCV was associated with a modest decrease in the fractional exposure of tryptophan to both iodide and acrylamide. 

The effect of phospholipid upon the conformation of apo-A-IV was investigated by fluorescence techniques sensitive to the exposure and electronic interaction of tyrosine and tryptophan residues. Apo-A-IV in Tris buffer displayed a maximum tryptophan fluorescence emission at 333 nm. The addition of a saturating concentration of EPCV to apo-A-IV solutions caused a blue shift in the wavelength of maximum fluorescence emission to 330 nm. In comparison, the tryptophan emission of apo-A-IV complexed in MC was blue-shifted only 1 nm to 332 nm. Fluorescence quenching studies revealed that binding of apo-A-IV to EPCV was associated with a modest decrease in the fractional exposure of tryptophan to both iodide and acrylamide. 

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**Fig. 4.** Excitation wavelength dependence of the relative tryptophan quantum yield of human apo-A-IV. The relative tryptophan quantum yield was determined as a function of excitation wavelength for pure apo-A-IV (C) and apo-A-IV bound to EPCV (A). The solid lines are best fits of the data and correspond to intramolecular tyrosine → tryptophan energy transfer efficiencies of 0.52 and 0.28. The dotted lines indicate the predicted excitation wavelength dependence of the relative tryptophan quantum yield when transfer efficiency is 1.00 (a) and 0 (b).

**Fig. 5.** Denaturation of human apo-A-IV and apo-A-IV/phospholipid recombinants by guanidine hydrochloride. The mean residue ellipticity at 222 nm ($\Theta_{222}$) was monitored as aliquots of buffered 8 M guanidine hydrochloride were added to solutions of pure apo-A-IV (O), apo-A-IV-phospholipid-cholesterol micellar complexes (A), and apo-A-IV bound to EPCV (■). Changes in $\Theta_{222}$ are expressed as percent of baseline value.

**Fig. 6.** Denaturation of human apo-A-IV and apo-A-IV-phospholipid recombinants by guanidine hydrochloride. Total fluorescence intensity between 290 and 370 nm was monitored as aliquots of buffered 8 M guanidine hydrochloride were added to solutions of pure apo-A-IV (O), apo-A-IV-phospholipid-cholesterol micellar complexes (A), and apo-A-IV bound to EPCV (■). Changes in fluorescence intensity are expressed as percent of baseline value.

**Fig. 7.** Determination of the free energy of stabilization of human apo-A-IV- and apo-A-IV-phospholipid recombinants. The data in Fig. 5 were analyzed according to Aune and Tanford (33). At each concentration of guanidine hydrochloride, the free energy of denaturation ($\Delta G^\circ$), calculated from the change in circular dichroism, is plotted against the chemical activity of guanidine hydrochloride. The y-intercept yields the free energy of stabilization in kcal/mol. O, pure apo-A-IV; □, apo-A-IV-phospholipid-cholesterol micellar complexes; A, apo-A-IV bound to EPCV.

**Discussion**

Our data establish that human apo-A-IV binds to phospholipid vesicles with an association constant in the same range as that of other soluble apolipoproteins (21, 31, 35, 36). The circular dichroism data demonstrate that binding to phospholipid induces the formation of additional α-helical structure in apo-A-IV. Such induction of α-helical structure has been observed for all the major apolipoproteins (21, 31, 35, 36) and is probably caused by coil → helix transformation in regions of random coil structure. Certainly, in apo-A-IV, there are several such regions which could assume an α-helical configuration under appropriate conditions (13).

The induction of α-helical structure is thought to provide the major thermodynamic driving force which stabilizes the association of apolipoproteins with lipid surfaces (21, 23, 31, 35, 36). The magnitude of this stabilization for apo-A-IV, greater than 6 kcal/mol, is comparable to values similarly determined for other HDL apolipoproteins bound to lipid (40, 41, 42, 46). Considering only this similarity, it might seem surprising that apo-A-IV competes so poorly with other apolipoproteins for the surface of plasma lipoproteins or phospholipid vesicles.
The rate of binding of apo-A-IV to EPCV as determined by the increase in fluorescence intensity was very rapid; similar rates have been observed for apo-A-I and apo-A-II (36, 48, 49). The increase in $Q_{22}$, however, was more gradual, and this suggests that the binding of apo-A-IV to EPCV may be a two-stage process: an initial rapid association of the protein with the EPCV surface, followed by a slower relaxation of the globular protein conformation in concert with the induction of $\alpha$-helical structure. Such coil-to-helix transformations may involve proline isomerization (50), and indeed, in apo-A-IV, proline residues punctuate several potentially inducible random coil domains (13, 51).

The fluorescence quenching and energy transfer data support such a mechanism. Binding to EPCV caused a profound decrease in the fractional exposure of tyrosine to iodide, and both fluorescence excitation techniques indicated that intramolecular tyrosine-tryptophan energy transfer in apo-A-IV decreased upon binding to EPCV. These findings suggest a conformational change that simultaneously increases shielding of tyrosine and increases the distance between tryptophan and tyrosine residues. We have proposed that the single tryptophan residue in apo-A-IV may reside in a domain formed by a confluence of tyrosine containing amphipathic $\alpha$-helices arranged with their hydrophobic faces oriented toward the interior of the protein (16). Thus, a plausible interpretation of the data is that during the second, slower phase of binding to the surface of EPCV, the tyrosine containing $\alpha$-helical domains surrounding the tryptophan "unfold" and reorient their hydrophobic faces toward the phospholipid monolayer, where they are shielded from iodide quenching by the high charge density of the phospholipid head groups and are stabilized by hydrophobic interaction with the vesicle surface. A similar mechanism has been proposed to explain the increased tyrosine fluorescence which accompanies the binding of apo-A-I to EPCV (52).

The binding of other apolipoproteins to lipid is accompanied by significant fluorescence emission blue shifts (21, 31, 37–39), which signal the transfer of tryptophan residues from an exposed aqueous environment to the hydrophobic shelter of the lipid monolayer. The observation that the binding of apo-A-IV to EPCV caused a very small blue shift, and only modest decreases in the accessibility of tryptophan to quenchers suggests that apo-A-IV is not deeply buried in the EPCV surface monolayer. The experiments with 6-carboxyfluorescein-loaded vesicles established that binding of apo-A-IV did not cause destabilization or breakdown in the vesicle structure, unlike the rapid disruption which occurs upon addition of other apolipoproteins (27, 53, 54). Since the mechanism by which surface-active proteins cause disruption of vesicles is postulated to require deep penetration of $\alpha$-helical domains into the phospholipid monolayer (42), this observation further suggests that apo-A-IV does not penetrate into the region of the phospholipid fatty acyl chains, but rather sits higher in the monolayer, intercalated between the charged phospholipid head groups.

Consideration of the stoichiometry of the binding of apo-A-IV to EPCV adds support to this hypothesis. As the EPCV had an average radius of 95 Å, the total surface area of each vesicle is $1.13 \times 10^5$ Å$^2$. Assuming that each vesicle contains 2013 phospholipid molecules of which 70% are located on the outer leaflet (23), and that the molecular area of each head group is 71 Å$^2$ (55), then the phospholipid head groups on each vesicle will occupy $1.00 \times 10^5$ Å$^2$, and the area potentially available for apolipoprotein binding is $1.32 \times 10^5$ Å$^2$. As apo-A-IV contains 376 amino acid residues (56), and as the average molecular area of each residue is 15.6 Å$^2$ (55), there would be space available between the phospholipid head groups of each vesicle for a maximum of 2.25 molecules of apo-A-IV. This calculated binding maximum agrees well with the observed binding maximum of 1.96 molecules of apo-A-IV per vesicle.

Incorporation of apo-A-IV into MC caused less induction of $\alpha$-helical structure, little fluorescence emission blue shift, less dramatic shielding of tyrosine residues, and less stabilization against denaturation. Hence, it is apparent that the interaction between apo-A-IV and phospholipid in these complexes had a smaller effect upon the structure and conformation of apo-A-IV. In apolipoprotein/MC, the apolipoproteins most likely bind circumferentially around the exposed edge of a bilayer phospholipid disk (57). Hence there may be insufficient lipid surface in the MC to accommodate unfolding and reorientation of the $\alpha$-helical domains of apo-A-IV. Alternatively, the apo-A-IV molecule may have insufficient hydrophobic surface for interaction with the fatty acyl chains of phospholipid molecules arrayed in a discoidal configuration.

The hypothesis that apo-A-IV does not penetrate to the region of the phospholipid fatty acyl chains when bound to a phospholipid surface could explain several aspects of its biochemical behavior. Apo-A-IV can activate lecithin-cholesterol acyltransferase, although it does so with only 20% of the efficiency of apo-A-I, the physiologic activator of lecithin-cholesterol acyltransferase. However, unlike apo-A-I, its catalytic efficiency is not reduced when phospholipids containing two saturated fatty acyl chains are used as a substrate (7, 8). Since the role of apolipoproteins in the lecithin-cholesterol acyltransferase reaction may be to increase the accessibility of phospholipid molecules to enzymatic attack by penetrating into the region of the fatty acyl chains on the lipid surface (58, 49), their catalytic efficiency should be sensitive to the physicochemical state of the phospholipid monolayer. If indeed apo-A-IV binds only superficially to phospholipid surfaces, then its catalytic efficiency for lecithin-cholesterol acyltransferase would be expected to be relatively insensitive to changes in the fluidity of the fatty acyl chains in the monolayer.

The in vitro action of lecithin-cholesterol acyltransferase causes a transient increase in the affinity and binding of human apo-A-IV to HDL (9, 60); a second, slower reaction, possibly related to lipid exchange phenomena (61), causes dissociation of apo-A-IV from the lipoprotein surface. If apo-A-IV binds only superficially to phospholipid surfaces, then its surface pressure necessary to exclude apo-A-IV from the HDL lipid monolayer should be considerably lower than that for apo-A-1 or apo-A-II (62, 63). Assuming that the resting pressure of the HDL surface is high enough to exclude apo-A-IV, depletion of surface phospholipid and cholesterol during the lecithin-cholesterol acyltransferase reaction would transiently reduce the surface pressure below the exclusion level for apo-A-IV, thus permitting binding of apo-A-IV to the HDL surface. Thereafter, the subsequent transfer of new lipid to the HDL surface by plasma lipid transfer proteins would restore the surface pressure above the exclusion point and cause the selective dissociation (64) of apo-A-IV.

An important biological implication of our data relates to the participation of apo-A-IV in reverse cholesterol transport. Discoidal lipoproteins composed primarily of phospholipid,
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free cholesterol, and apo-A-IV have been observed in the peripheral lymph of cholesterol-fed animals (65, 66) and in the serum of patients with lecithin-cholesterol acyltransferase deficiency (67); small vesicular lipoproteins containing apo-A-IV have been identified in the HDL density range of fasting human serum (20). Sloop et al. (68) have proposed that formation of such complexes may be the earliest step in reverse cholesterol transport. Our observation that the binding of apo-A-IV to phospholipid is energetically favorable supports a role of apo-A-IV in reverse cholesterol transport, for the stabilization of apo-A-IV structure upon binding to phospholipid could provide the thermodynamic driving force for the efflux of cellular membrane lipids by free apo-A-IV present in the pericellular lymph. Moreover, since concentrations of GdmCl as low as 0.25M dissociate dimeric apo-A-IV into monomers (17), the denaturation data imply that apo-A-IV bound to phospholipid is more stable than dimeric apo-A-IV. Hence, binding of apo-A-IV to phospholipid in vivo would result in the dissociation of circulating dimers (17) and an increase in the pool of monomeric apo-A-IV able to filter into the lymphatic compartment.

In summary, the binding of apo-A-IV to phospholipid vesicles results in the induction of α-helical structure and an increase in molecular stability. Nonetheless, apo-A-IV may have a limited ability to penetrate deeply into the surface of phospholipid monolayers, and this property may determine its labile interaction with HDL and its unusual mode of lecithin-cholesterol acyltransferase activation.

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