The Structure of Human Lipoprotein A-I

**EVIDENCE FOR THE “BELT” MODEL***

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Vishwanath Koppaka‡, Loraine Silvestro*, Jeffrey A. Engler§, Christie G. Brouillette‡‡, and Paul H. Axelsen‡‡‡

From the ‡Department of Pharmacology, ©Department of Medicine, Infectious Diseases Section, and the Johnson Foundation for Molecular Biophysics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104-6084 and the ©Department of Biochemistry and Molecular Genetics and ¶Department of Physiological Optics, Center for Macromolecular Crystallography, University of Alabama at Birmingham Medical Center, Birmingham, Alabama 35294

The two main competing models for the structure of discoidal lipoprotein A-I complexes both presume that the protein component is helical and situated around the perimeter of a lipid bilayer disc. However, the more popular “picket fence” model orients the protein helices perpendicular to the surface of the lipid bilayer, while the alternative “belt” model orients them parallel to the bilayer surface. To distinguish between these models, we have investigated the structure of human lipoprotein A-I using a novel form of polarized internal reflection infrared spectroscopy that can characterize the relative orientation of protein and lipid components in the lipoprotein complexes under native conditions. Our results verify lipid bilayer structure in the complexes and point unambiguously to the belt model.

Human apolipoprotein A-I (apoA-I) comprises 70% of the total protein found in plasma high density lipoproteins. As a lipoprotein complex, apoA-I is thought to mediate a variety of functions, including (i) the binding and transport of plasma lipids, (ii) activation of lecithin-cholesterol acyltransferase (1), (iii) stimulation of cholesterol efflux from peripheral tissues (2, 3), and (iv) uptake by the liver via a human apoA-I-specific receptor (4, 5). The later two processes have been called “reverse cholesterol transport” and may account for the inverse relationship observed between plasma high density lipoprotein concentration and the risk of developing coronary artery disease (6–8).

Sequence analysis suggested that apoA-I forms a series of amphipathic α-helices (9), and this has been confirmed in the crystal structure of a lipid-free amino-terminal truncation mutant of human apoA-I (apoA1–43A-I) (10). However, it is not clear which of two disparate models best describes the orientation of these helices within the lipoprotein complex. One model positions the helices of apoA-I around the perimeter of a lipid bilayer disc and orients them perpendicular to the plane of the bilayer. This “picket fence” model (Fig. 1, right) (11, 12) is supported primarily by an internal reflection infrared spectroscopy study of dried lipoprotein films (13). The “belt” model (Fig. 1, left), on the other hand, also positions the helices of apoA-I around the perimeter of a lipid bilayer disc, but orients them parallel to the plane of the bilayer (14–16). The results of Borhani et al. (10) and Rogers et al. (17, 18) are more consistent with the belt model.

We have examined the orientation of protein and lipid components in these lipoprotein complexes using specialized instrumentation for performing polarized attenuated total internal reflection infrared spectroscopy on supported lipid monolayers (19–24). In the presence of 1 mM Ca2+, lipoprotein complexes adsorb to supported monolayers and permit one to record polarized internal reflection infrared absorption spectra of oriented complexes (Fig. 1). Since the complexes remain in buffer throughout the measurement, this approach permits a direct spectroscopic study of uniformly oriented lipoprotein complexes under native conditions (25).

EXPERIMENTAL PROCEDURES

The 243-residue wild type apolipoprotein A-I (apoA-I) was purified from human plasma, and a 200-residue NH2-terminal deletion mutant (apoA1–43A-I) was expressed and purified as described previously (26–28). Lipid-free apoproteins were prepared for study by dissolving them in 6 M guanidine hydrochloride, 20 mM Hepes, and 150 mM NaCl at pH 7.4, then extensively dialyzed using tubing with a 5,000-dalton cutoff to remove the guanidine. Following this, they were dialyzed against a D2O-based buffer (30 mM Hepes, 150 mM NaCl, pH 7.4). Protein concentrations were determined in 6 M guanidine hydrochloride by absorption at 280 nm using extinction coefficients of 1.13 and 0.992 ml/(mg cm) for apoA-I and apoA1–43A-I, respectively. Lipoprotein complexes were reconstituted as discoidal lipoproteins using synthetic dimyristoylphosphatidylcholine (DMPC) and dimeristoylphosphatidylserine (DMPS) from Avanti Polar Lipids, Inc. (Alabaster, AL). The lipid was a mixture of DMPC:DMPS at a 9:1 molar ratio in organic solution, dried with a stream of nitrogen, and then further dried under vacuum for at least 4 h (15). The lipids were resuspended with proteins and 8 M guanidine hydrochloride in PBS buffer (0.02 M sodium phosphate, 0.15 M sodium chloride, pH 7.4) and diluted with PBS to a protein concentration of ~1 mg/ml. This sample was gently shaken at room temperature for 3–5 h and dialyzed exhaustively against PBS with a 12–14-kDa molecular mass cutoff membrane, followed by dialysis against 30 mM Hepes, pH 7.4, with a 50-kDa molecular mass cutoff membrane to remove excess free protein. This procedure produced homogeneously sized particles with two to three discrete complexes between 10 and 13 nm in diameter as confirmed by native gradient gel electrophoresis. Homogeneously sized particles were produced by detergent dialysis (27), followed by gel filtration to remove excess free protein. Prior to spectroscopic study, the optically clear solution was dialyzed against 100 ml of 30 mM Hepes, pH 7.4, D2O buffer. This procedure yielded homogeneously sized particles of approximately 7.8 nm in diameter that migrated as a single band before and after prolonged exposure to 1 mM Ca2+. The concentrations of DMPS used in these experiments are well below the threshold at which nonliquid lipid phases would be induced by 1 mM Ca2+ (29).

Polarized attenuated total internal reflection Fourier-transform (PATIR-FTIR) spectroscopy was performed on a previously described instrument (19), modified such that the internal reflection crystal is
applied flat onto a monolayer at the air-water interface in a Langmuir trough (23). The angle of incidence between the IR beam and the crystal surface was 30°. The internal reflection crystal was treated with octadecyltrichlorosilane to render the surface hydrophobic (19, 30). All spectra (1024 co-added interferograms) were collected at room temperature, using a Bio-Rad FTS-60A spectrometer in rapid-scanning mode, a liquid-nitrogen-cooled MCT detector, a resolution of 2 cm⁻¹, triangular apodization, and one level of zero filling. Base-line single-beam spectra were recorded immediately before the addition of 10 μg of lipoprotein complex or lipid-free apoprotein to a continuously stirred buffer subphase (30 mM HEPES in D₂O, pH 7.4). The volume of the subphase was 6 ml, making the surface area 90 cm², the signal plateau most likely corresponds to adsorption of the entire injected sample onto the monolayer, and it is unlikely the lipoprotein complexes have “stacked” on the support membrane. Irrespective of whether the complexes are stacked, however, conclusions drawn below with respect to the relative orientation of protein and lipid components remain valid.

Representative infrared spectra of the hA-1 lipoprotein complex are shown in Fig. 2, a and b; spectra of the Δ(1–43)A-1 complex are similar. The lipid components are seen as bands at 2920 cm⁻¹ (Eq. 2) and 2850 cm⁻¹ from the symmetric CH₂ stretch, at 1740 cm⁻¹ from the ester C = O stretch. Amide I from the protein component is maximal at 1645 cm⁻¹, consistent with an α-helical conformation, while amide II at 1550 cm⁻¹ (off scale in Fig. 2b) is not detectable. The absence of an amide II band at 1550 cm⁻¹ indicates that H → D exchange is virtually complete.

When lipid-free apohA-I or apΔ(1–43)A-1 was introduced into a subphase buffer containing 1 mM Ca²⁺, an amide I signal developed over a similar period of time as did the lipoprotein complexes, and their amplitudes were comparable with those of the complexes. The amide I absorption maximum occurred at 1645 cm⁻¹, but there were no characteristic lipid absorption signals (Fig. 2c). Bands originating from the supported monolayer are not seen because they are part of the background spectrum collected prior to introduction of the complexes.

When calcium was omitted from the subphase, the signal arising after the introduction of lipoprotein complexes into the subphase was negligible. This demonstrates that freely diffusing complexes do not contribute to the spectroscopic signal. In contrast, omitting Ca²⁺ from the subphase buffer reduced the signal from the apoproteins by only 50%. This is important control information, because it indicates that the preparation of lipoprotein complexes used in these experiments is effectively free of apoprotein. If this were not true, we would have detected an amide I absorption when the lipoprotein complexes were examined in the absence of Ca²⁺.

From the polarized absorption spectra, dichroic ratios \( R_z = \frac{\int A_{∥ \parallel} d\lambda}{\int A_{∥ \perp} d\lambda} \) were evaluated using integrated areas of characteristic lipid absorption bands, \( A \), as described elsewhere (24). Dichroic ratios were converted to order parameters, \( S(\psi) \), according to Equations 1–3.

\[
S(\psi) = \frac{(E_E) - R_2(E_E) + (E_H)}{(E_E) - R_2(E_E) - 2E_H} \tag{1}
\]

\[
S(\psi) = \frac{(P_0\cos \theta)/P_0(\cos \gamma)}{P_0(\cos \theta)} \tag{2}
\]

\[
S(\psi) = S_2S_3S_4 \tag{3}
\]
Angle brackets indicate mean values, \( P_2(x) = (3x^2 - 1)/2 \) is the second-order Legendre polynomial, which relates to order parameters according to \( S_x = \langle P_2(\cos x) \rangle \), \( \theta \) is the angle between the molecular axis and the vibrational transition moment, \( \gamma \) is the angle between the molecular axis and the surface normal, and \( \xi \) is the angle representing the orientation of the monolayer surface with respect to the crystal surface (the mosaic spread) (30). An order parameter of 1.0 indicates a uniform orientation perpendicular to the membrane surface, while a value of \(-0.5\) indicates a uniform orientation parallel to the membrane. An order parameter of 0.0 may indicate either a uniform orientation at the magic angle (54.7° relative to the surface normal), complete disorder as in an isotropic system, or any other orientation distribution for which \( \langle \cos^2 \gamma \rangle = 1/3 \).

\( S(R_j) \) values between \(-0.45\) and \(-0.41\) were obtained for the symmetric methylene stretching mode at \(-2850 \text{ cm}^{-1} \) in the lipoprotein complexes (Table I). \( S_y = -0.5 \), because this mode is oriented 90° from the acyl chain axis, and \( S_y < 1.0 \), because there will always be some mosaic spread due to imperfections in the crystal support. Therefore the molecular order of the lipid acyl chains in the complexes \( S_y = S(R_j)/S_x(S_j) \geq 0.82 \). This is close to the maximum possible value of 1.0, and it indicates that the disceoidal lipoprotein complexes are highly ordered on the monolayer surface with their acyl chains oriented perpendicular to the surface. In the same spectra, we find that the antisymmetric methylene stretching mode (at 2920 cm\(^{-1}\)) is also highly ordered and similarly oriented.

\( S(R_j) \) was \(-0.19\) for the amide I band at \(-1650 \text{ cm}^{-1} \) in the heterogeneous apohA-I complexes, \(-0.22\) for the apo\(\Delta(1–43)\)A-I complexes, and \(-0.28\) for the homogeneously sized small complexes (Table I). The difference between the heterogeneous apohA-I complexes and the homogeneously sized small complexes is statistically significant, and it suggests that the first 43 residues of apohA-I may not be aligned with the remainder of the protein. The difference between the heterogeneous and the homogeneous apo\(\Delta(1–43)\)A-I complexes is not statistically significant, and differences in the order of the methylene stretching modes suggests that the appearance of some change in amide I transition order may be due to the smaller complexes simply being more uniformly oriented. Estimates of \( S_j \) range from 0.53 to 0.83 for right-handed \( \alpha \)-helices (30), and \( S_j > 0.82 \) from the preceding paragraph. Therefore \(-0.5 \leq S_j \leq -0.28 \) for \( \alpha \)-helices in the heterogeneous complexes, and \(-0.5 \leq S_j \leq -0.34 \) for the smaller homogeneous complexes.

When molecular orientations are uniform, order parameters may be re-interpreted as "tilt angles" (30). This view assigns a tilt angle between 0° and 20° from the membrane normal to the lipid acyl chains and between 67° and 90° to the protein helices.

**DISCUSSION**

These results have several important implications. First, they show that lipid molecules within these complexes are highly ordered, as in a bilayer membrane with its surface oriented parallel to the monolayer support. Second, they show that the protein component of these complexes is preferentially oriented parallel to the surface of the lipid bilayer as in the belt model (Fig. 1, left). Third, they show that complexes made with apo\(\Delta(1–43)\)A-I (for which a crystal structure is available) yield results that are similar to those made with apohA-I. The apo\(\Delta(1–43)\)A-I crystal structure is a homotetramer of predom-

![FIG. 2. PATIR-FTIR spectra of methylene stretching bands of the lipoprotein hA-I complex (a), lipid ester and protein amide I bands of the lipoprotein hA-I complex (b), and the amide I band of apoprotein hA-I (c).](image)

In each panel, the upper spectrum (solid line) is parallel polarized, and the lower spectrum (dotted line) is perpendicular polarized. Absorption due to the supporting monolayer is part of the background spectra, and therefore it does not contribute to these spectra. Likewise, there are no spectral changes attributable to lipid components when 1 mM Ca\(^{2+}\) is added in the absence of lipoprotein complexes or when lipid-free apoprotein hA-I adsorbs to the surface.

**TABLE I**

**Order parameters obtained for the apoproteins and their lipid complexes**

Order parameters were calculated using the "two-phase" approximation because the proteins are not actually embedded within a thin film, and because it yields results that are numerically identical to the "thin film" approximation for methylene stretching modes when they lie in the plane of the membrane (20, 21). For this instrument, the isotropic dichroic ratio \( R_{ISO} \), the ratio for which \( S(R) = 0 \) using the two phase approximation is 2.33. If the thin film approximation (33) is applied, \( R_{ISO} = 1.86 \), and the amide I order parameters are decreased (e.g. the tilt angle for the heterogeneous hA-I complexes becomes 55°–90°). This does not, however, change any principal conclusions.

| Vibrational band                  | Heterogeneous apo\(\Delta(1–43)\)A-I complexes | Heterogeneous apo\(\Delta(1–43)\)A-I complexes | Homogeneous small apo\(\Delta(1–43)\)A-I complex |
|-----------------------------------|---------------------------------------------|---------------------------------------------|-----------------------------------------------|
| Amide I (\( \approx 1645 \text{ cm}^{-1} \)) | \( R_y = 1.62 \pm 0.12 \) | \( S(R) = -0.19 \pm 0.04 \) | \( S(R) = 0.95 \pm 0.02 \) |
| Tilt angle of helix axis          | 67°–90°                                     | 70°–90°                                     | 71°–90°                                       |
| CH\(_2\) symmetric stretch (\( \approx 2852 \text{ cm}^{-1} \)) | \( R_y = 0.98 \pm 0.11 \) | \( S(R) = -0.43 \pm 0.05 \) | \( S(R) = 0.05 \pm 0.01 \) |
| Tilt angle of acyl chain          | 0°–18°                                      | 0°–20°                                      | 0°–15°                                        |
nantly helical amphiphilic segments in the shape of a bent elliptical ring. With simple and plausible manipulations, one may transform a pair of these segments into a circular ring (10) with a hydrophobic inner circumference and a diameter of 7.8 nm (the same as that of the small homogeneously sized complexes examined in this report). A ring of this size is well suited to encircle 60 lipid molecules per protein segment in the manner prescribed by the belt model and to explain all of the experimental results reported herein.

Our measurements were made on complexes under conditions that do not denature the protein or disrupt the complexes (25). In contrast, the earlier study of apolipoprotein A-I on which the picket fence model has been based was performed on complexes that had been dried onto a crystal surface as a multibilayer film (13). By drying the complexes, one forfeits control over conditions such as ionic strength and pH and risks denaturing the protein and/or disrupting the complexes, because the hydrophobic effect is largely responsible for their stability. Although the film is partially rehydrated by exposure to water vapor, relatively few water molecules are actually present (32), and preparations of this type yield spectroscopic results that differ from lipid monolayers and bilayers in ways that suggest denaturation and/or aggregation (22).

In summary, our results unambiguously support the belt model of lipoprotein A-I structure and refute the picket fence model (Fig. 1). The data were obtained using an approach that combines internal reflection infrared spectroscopy and Langmuir film balance technology and is advantageous because it provides an abundance of quantitative structural information about protein-lipid interactions under conditions that are unlikely to disrupt the complexes or denature the protein component.

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