Lipid association is a prerequisite for receptor interactions of apolipoprotein E (apoE). Disc complexes of the N-terminal 22-kDa apoE3 receptor binding domain and dimyristoylphosphatidylcholine display full receptor binding activity. Studies have been performed to characterize conformational adaptations of the globular, lipid-free four-helix bundle structure that culminate in stable association of its amphipathic α-helices with a lipid surface. Helix-lipid interactions in bilayer disc complexes can conceivably adopt two orientations: parallel or perpendicular to the phospholipid acyl chains. Evidence based on infrared dichroism, geometrical arguments, and x-ray crystallography support the view that defined helical segments in the four-helix bundle realign upon lipid association, orienting perpendicular to the phospholipid fatty acyl chains. Evidence that defined helical segments align in tandem, presenting a convex receptor-active surface.

Apolipoprotein E (apoE) is an important plasma protein that functions in triacylglycerol and cholesterol transport. In addition to its structural role in stabilization of circulating lipoproteins, apoE serves as a ligand for at least three lipoprotein receptors, the low density lipoprotein (LDL) receptor, the LDL receptor-related protein, and the very low density lipoprotein receptor (1). By directing the cellular uptake of circulating lipoproteins, apoE functions in maintenance of plasma lipid homeostasis. The critical role played by apoE in this process is vividly seen in apoE knockout mice, which are predisposed to hypercholesterolemia and atherosclerosis (2, 3). More recently, the E4 isoform has been demonstrated as a major genetic risk factor in the predisposition to Alzheimer’s disease (4–6).

ApoE is composed of two independently folded structural domains (7). The C-terminal domain (residues 216–299) is known to have a high lipid binding affinity, whereas the N-terminal domain (residues 1–191) harbors the receptor binding region (8, 9). Upon thrombolytic cleavage, it is possible to separate the two domains (7), which retain structures similar to those present in the intact protein (10). The three-dimensional structure of the 22-kDa N-terminal domain has been determined by x-ray crystallography (11), revealing an elongated globular four-helix bundle. Individual helices (up to 37 amino acids) are distinctly amphipathic and orient with their hydrophobic faces directed toward the center of the bundle. This molecular architecture, which resembles the structure of invertebrate apolipopophorin III (12, 13), provides a structural basis for the water solubility of this protein in its lipid-free state.

Like most exchangeable apolipoproteins, the N-terminal domain of apoE is capable of transforming multilamellar vesicles of dimyristoylphosphatidylcholine (DMPC) into uniform-size bilayer disc complexes. Interestingly, in the absence of lipid, the receptor binding region (residues 130–150; Ref. 9) does not interact with the LDL receptor on fibroblasts (14). Thus, it is postulated that lipid binding induces a conformational change in this domain, which confers receptor recognition properties. A description of the structure and association of apoE N-terminal domain DMPC disc complexes, which mimic lipoprotein complexes containing the full-length apoE in terms of receptor binding interactions (8), is of primary importance in terms of understanding the molecular details of this process.

We have used FTIR-ATR spectroscopy to study the N-terminal domain (residues 1–183) of the apoE3 isoform. First, we evaluated the secondary structure of the protein, both free in solution and complexed with DMPC, and compared it with previous measurements (10, 15) and with the x-ray structure of the lipid-free protein (11). Second, using linear dichroism, we provide experimental evidence that apoE3 N-terminal domain α-helices orient perpendicular to the acyl chains of the lipids in discoidal complexes. Based on these dichroism IR data and geometric considerations, we propose a model of association of the apoE3 N-terminal domain in discoidal complexes and discuss possible implications in terms of the apoE3 structure-function relationship.

EXPERIMENTAL PROCEDURES

Materials—DMPC was purchased from Sigma. Phospholipid (cholesterol) enzymatic colorimetric analysis kit was obtained from Wako Pure Chemical Industries.

Preparation of Dimyristoylphosphatidylcholine Disc Complexes—Dimyristoylphosphatidylcholine disc complexes were prepared by the cholate dialysis method.
ApoE Conformation in Disc Particles

(17). Briefly, 2 mg of DMPC, dissolved in CHCl₃, was dried under N₂ to form a thin film. 90 µl of prewarmed (37°C) sodium cholate (30 mg/ml) was added to create a lipid suspension. The contents were incubated at 37°C with shaking for 30 min, vortexing every 10 min. Subsequently, 0.9 mg of recombinant apoE3(1–183) and buffer were added (final buffer concentration 10 mM Tris, pH 7.4, 140 mM NaCl, 150 nM NaN₃, 250 nM EDTA), and the mixture was incubated at 37°C for 1 h with shaking. The solution was dialyzed against buffer to remove the cholate. Where necessary, free apolipoprotein was removed by gel permeation chromatography on a Sepharose CL-6B column.

Disc Particle Characterization—ApoE3(1–183):DMPC complexes were subjected to non-denaturing gradient gel electrophoresis on a 4–20% acrylamide slab with Stokes diameter determined by comparison with the mobility of known standards (18). Samples for microscopy were adsorbed to carbon-coated grids and negatively stained with 2% sodium phosphotungstate, pH 7.0 (19). Grids were photographed in a Philips EM 420 operated at 100 kV. Protein assays were performed using the bicinchoninic acid assay (Pierce) using bovine serum albumin as the standard. Phospholipid analysis was carried out using an enzyme-based colorimetric assay.

Analytical Ultracentrifugation—A Beckman model E analytical ultracentrifuge was used for hydrodynamic analysis of apoE3(1–183):DMPC complexes. Samples were dialyzed for 24 h against 100 mM Tris, pH 7.5, 2.5 mM KBr to equilibrate the complexes at a density of 1.21 g/ml. The Rayleigh interference optical system was used for flotation equilibrium experiments using the high speed equilibrium technique described by Nelson et al. (20). Runs were performed at 18,000 rpm at 293 K in a double sector, charcoal-etched Epon sample cell equipped with sapphire windows for a minimum of 48 h before equilibrium photographs were taken. Molecular mass calculations were carried out using an APL computer program. The ln Y versus r² data were fitted to a second order polynomial equation using least squares techniques, and the apparent weight average molecular mass was calculated from the slope of the resulting plot.

IR Spectroscopy—Spectra were recorded on a Bruker IFS 55 infrared spectrophotometer equipped with a reflection accessory and a polarizer mount assembly with a gold wire grid element. The internal reflection element was a germanium ATR plate (50 × 20 × 2 mm, Harrick Scientific) with an aperture angle of 45° yielding 25 internal reflections. 256 accumulations were performed to improve the signal/noise ratio. The spectrophotometer was continuously purged with air dried on a silica gel column (5 × 130 cm). Spectra were recorded at a nominal resolution of 2 cm⁻¹. All the measurements were made at 30°C. Prior to any analysis, the side-chain contributions to the spectra were subtracted according to Groomaghtigh et al. (21).

Sample Preparation—Oriented multilayers were formed by slow evaporation of ~50 µl of the sample (~2 mg/ml) on one side of the ATR plate. The ATR plate was then sealed in a universal sample holder.

Secondary Structure Determination—Secondary structure quantification was performed on samples subjected to 1 h of deuteration as described previously (22). Briefly, Fourier self-deconvolution was applied to obtain resolution of the spectra in the amide I region. Least squares iterative curve fitting was performed to fit different components of the amide I band revealed by the self-deconvolution to the non-deconvoluted spectrum between 1700 and 1660 cm⁻¹. The proportion of various structural elements was computed as reported (22).

Orientation of the Secondary Structures—For the amount of material used in the present studies, film thickness remains small when compared with the IR wavelength. This allows the “thin film” approximation to be used for establishment of equations describing the dichroic ratio as a function of the orientational order parameter (23). In an α-helix, the main transition dipole moment lies approximately parallel to the helical axis. It is therefore possible to determine the mean orientation of the α-helix structure from the orientation of the peptide bond C=O group (23). To obtain this information, additional spectra were recorded with parallel and perpendicular polarized incident light with respect to a normal to the ATR plate. Polarization was expressed as the dichroic ratio Rₜₜ = Aₜ/4A. The mean angle between the helix axes and a normal to the ATR plate surface was then calculated from Rₜₜ. In these calculations, a 27° angle between the long axis of the α-helix and the dipole moment was considered (23, 24). The γ₁(CH2) transition at 1201 cm⁻¹, whose dipole lies parallel to the all-trans hydrocarbon chains, was used to characterize the lipid acyl chain orientation (25).

When two helix populations (or any other structure) are present in a sample, both orientations must be considered in the interpretation of the dichroic ratio. We define the dichroic ratios of helix populations a and b as follows:

\[ R_{\alpha} = \frac{A_{\alpha}}{A_{\alpha} + 2A_{\beta}} \]  
(Eq. 1)

\[ R_{\beta} = \frac{A_{\beta}}{A_{\alpha} + 2A_{\beta}} \]  
(Eq. 2)

and the experimental dichroic ratio is given as follows:

\[ R_{exp} = \frac{A_{\alpha} + 2A_{\beta}}{A_{\alpha} + 2A_{\beta}} \]  
(Eq. 3)

Because the total, unpolarized contribution from populations a and b must remain proportional to the fraction of population a (x) and b (1 – x), the following must be true:

\[ x = \frac{A_{\alpha} + 2A_{\beta}}{A_{\alpha} + 2A_{\beta}} \]  
(Eq. 4)

Resolving these equations, we find the following results:

\[ R_{\alpha} = \frac{x(R_{\alpha} + 2) + 2(1 - x)(R - R_{\alpha})}{x(R_{\alpha} + 2) + (1 - x)(R - R_{\alpha})}, \]  
(Eq. 5)

\[ R_{\beta} = \frac{(1 - x)(R_{\beta} + 2) + 2x(R - R_{\beta})}{(1 - x)(R_{\beta} + 2) + x(R - R_{\beta})}, \]  
(Eq. 6)

\[ x = \frac{(R - R_{\alpha})(R_{\alpha} + 2) - (R_{\alpha} + 2)(R - R_{\beta})}{(R_{\alpha} + 2)(R - R_{\alpha}) - (R_{\alpha} + 2)(R - R_{\beta})} \]  
(Eq. 7)

RESULTS AND DISCUSSION

ApoE3(1–183):DMPC Disc Particles—The cholate dialysis method was employed to prepare apoE3(1–183):DMPC disc complexes. Non-denaturing gradient gel electrophoresis indicated the presence of a uniform population of complexes. Comparison of the relative migration of the complexes versus that of known standards yielded a Stokes diameter of ~11 nm. The morphology of the complexes was investigated by negative stain electron microscopy, revealing disc-shaped complexes of a similar diameter. Subsequently, flotation equilibrium experiments were conducted to evaluate the molecular mass of the apoE3(1–183):DMPC discs. Plots of ln Y versus r² yielded a straight line, indicating one species of complex was present with an apparent molecular weight of 468,000. Compositional analysis of the discs revealed a molar ratio of DMPC:apoE3(1–183) of ~200:1.

Secondary Structure Characterization—Fig. 1 displays the IR spectra of a lipid-free apoE3(1–183) sample (bottom spectrum) and apoE3(1–183):DMPC discoidal complexes (upper spectrum) in the region of 1800–1400 cm⁻¹. The amide I band (1700–1600 cm⁻¹) reveals a sharp peak centered at 1654 cm⁻¹ for lipid-free apoE3(1–183) and at 1653 cm⁻¹ for the apoE3(1–183):DMPC sample. 1654 and 1653 cm⁻¹ are wave numbers characteristic of α-helical structures (24, 26). The maximum intensity of the amide II peaks (1600–1500 cm⁻¹) are located at 1547 cm⁻¹, a region normally assigned to helical structures (24, 26). Quantification of secondary structure conformers present was performed on samples subjected to deuteration for 1 h. Partial deuteration of the sample allows for discrimination between the relative contributions of random coil and α-helix. The percentage of helical secondary structure reaches 65% for lipid-free apoE3(1–183) and 66% for the protein in complex with DMPC (Table 1). Similar amounts of other secondary structure elements were observed in both lipid-free and DMPC-bound apoE3(1–183). Conceivably, these arise from the extreme N- and C-terminal regions that were disordered in the crystal (11).

The 65% α-helix content determined for apoE3(1–183) by ATR-FTIR spectroscopy is in good agreement with data obtained by CD and FTIR (10, 15). Using the DSSP algorithm (27)
to analyze the x-ray atomic coordinates of the protein in the protein data base file (1lpe), five discrete α-helical segments were identified. Four of these, designated H1–H4, constitute the bundle and range in length from 17 to 37 residues, whereas a fifth short helix, H* (residues 45–52), connects H1 and H2 and orients perpendicular to the bundle axis. Thus, 118 of the 183 amino acids present are in a helical conformation (64.5%). Thus, values derived from FTIR analysis (65%) are in very good agreement with corresponding values determined from the x-ray crystal structure.

ApoE3(1–183) complexed with DMPC was found to possess 60% α-helical structure. Although this value is in agreement with data obtained by CD (10), it is higher than that reported by De Pauw et al. (15) on the basis of CD and FTIR measurements. Our data suggest that there is no real change in the secondary structure content in the N-terminal domain of apoE3 upon binding to lipid with subtle changes observed, likely reflecting a change in tertiary structure.

**Determination of the Orientation of the Helices**—Spectra of apoE3(1–183):DMPC complexes were recorded with parallel and perpendicular polarized light (Fig. 2, A and B, respectively; see Ref. 23 for the geometry of the experiment). The dichroic spectrum (Fig. 2C) was obtained by subtracting the spectrum recorded with perpendicular polarized light from the spectrum recorded with parallel polarized light. Initially, the orientation of DMPC acyl chains in disc complexes was assessed using characteristic bands associated with lipid molecules. Specific spectral bands, such as ν(CH2) at 2916 cm−1, ν(CH3) at 2849 cm−1, and δ(CH2) at 1467 cm−1, whose dipoles orient perpendicular to the hydrocarbon chains of DMPC (25), displayed a negative deviation in the dichroic spectrum (Fig. 2C). This indicates an orientation perpendicular to the normal of the germanium crystal plane. Conversely, bands corresponding to dipoles that orient parallel to the hydrocarbon chains (ν(CH3) at 2962 cm−1 and the bands of the γ(CH2) progression observed for saturated hydrocarbon chains with all-trans configuration at 1328, 1306, 1279, 1260, 1229, and 1201 cm−1) had a positive deviation in the dichroic spectrum. Thus, these orient parallel to the normal of the germanium plane. As described

**FIG. 2. IR spectra of apoE3(1–183):DMPC complexes.** Spectrum A was obtained with parallel polarized light and spectrum B with perpendicular polarized light. Spectrum C is the dichroic spectrum obtained by subtracting spectrum B from spectrum A. The optical density amplitude of spectrum C has been increased five times with respect to spectra A and B. Measurements were made at 30 °C.
Previously (28), we used the last peak in the $\gamma_a$(CH$_2$) progression peak (1201 cm$^{-1}$) to quantify the lipid chain orientation. The measured dichroic ratio for this band is 4.45 with an isotropic dichroic ratio of 1.67. On the basis of these dichroic ratio measurements, we can assume that the maximum tilt between the acyl chains and a normal to the germanium surface is 15–20°. As any additional source of disorder would reduce this tilt (28), the value of 15–20° is a maximum tilt with respect to a normal to the germanium plate. By defining the orientation of a DMPC disc by a normal vector passing through the face of the disc, the results show that the normal vector is oriented parallel to a normal to the germanium plate.

In the case of apoE3(1–183), helix orientation was determined using the amide I band. In the dichroic spectrum (Fig. 2C), a strong negative deviation at 1648 cm$^{-1}$ is observed, indicating a parallel orientation of the associated dipole to the surface of the Ge plate and, thus, a perpendicular orientation with respect to a vector normal to the face of the disc. From the secondary structure of apoE3(1–183), and the frequency of the negative deviation (1648 cm$^{-1}$), we conclude that the dipole responsible for this deviation is associated with $\alpha$-helices, which orient in the direction of the helical axes (24). Thus, the negative deviation observed indicates that the helices are primarily oriented perpendicular to the normal vector of the disc and, therefore, perpendicular to the hydrocarbon chains of the lipids. This helix orientation is confirmed by two additional observations: 1) negative deviation of the amide I band at 3282 cm$^{-1}$, for which the dipole orientation is similar to that for the amide I band, and 2) positive deviation of the amide II band (at 1547 cm$^{-1}$), which is oriented in the opposite, perpendicular direction with respect to the helical axes (see Refs. 23 and 24 for a complete discussion of orientation determination in ATR-FTIR).

To quantitatively determine the orientation of the apoE3(1–183) helical segments in the disc complex, we decomposed and curve-fitted the amide I region of the parallel and perpendicular polarized light spectra (Fig. 2, A and B, respectively) with four Lorentzian curves (Fig. 3). Using the main curve associated to the helices (maximum intensity at 1652 cm$^{-1}$), we calculated a dichroic ratio associated with the helical structure in the protein equal to 1.25. With such a dichroic ratio, and assuming that the normal to the disc is parallel to the normal to the plane (see above), the minimum mean tilt between the helical axes and a normal to the face of the DMPC disc is 60–65°.

From the x-ray crystal structure of apoE3 N-terminal domain (11), it is recognized that helix H$^*$ (the 8 residue helix that connects helix 1 and helix 2 in the bundle) orients perpendicular to the four long helices. Thus, helical segments in the bundle can be separated into two groups with different orientations. Using the equations described under “Experimental Procedures,” we found that the 1.25 dichroic ratio could also be decomposed into two components with different orientations. A first component representing 92% of the helix content has a 1.11 dichroic ratio, whereas the second, representing the rest of the helix content, has a dichroic ratio of 4.5. It seems reasonable that the 1.11 ratio representing 92% of the helices could be attributed to the four long helices (representing 110 amino acids of a total of 118 in helix conformation), which orient perpendicular to a vector normal to both the face of the disc and the germanium plate (65–70° minimum tilt). On the other hand, the dichroic ratio of 4.5 could be attributed to helix H$^*$, suggesting this helix orients parallel to the normal vector of the disc, with a maximum tilt of 10–15°.

**Model of Association**—Our results using linear IR dichroism to characterize the orientation of apoE3(1–183) helical segments when bound to DMPC bilayer discs are consistent with a model wherein the four main helices (H1–H4) orient perpendicular with respect to a normal to the face of the discs (and the DMPC acyl chains), whereas helix H$^*$ adopts the opposite orientation. Fig. 4 depicts a model of apoE3(1–183):DMPC discoidal particles in which the helices of the protein align around the periphery of the discoidal phospholipid bilayer, as previously proposed and experimentally demonstrated for insect apolipoporin III (28, 29). In solution, the hydrophobic side chains of the amphipathic helices of apoE3(1–183) are oriented toward the interior of the bundle (11). It has been suggested that, upon lipid interaction, the hydrophobic faces of the helices interact with the hydrophobic acyl chains (9). This could be accomplished by a conformational change in which the bundle opens, without disruption of the helical boundaries, by pivoting around a hinge region connecting H2 and H3, as proposed by Weisgraber (9). This “open” conformation would expose the hydrophobic faces of individual helices, permitting their direct contact with the lipid surface, specifically phospholipid acyl chains around the perimeter of the disc. In this conformation, the hydrophobic faces of the helices retain contact with the aqueous environment, whereas the hydrophobic faces essentially substitute helix-lipid interactions for the helix-helix interactions which stabilize the bundle (11).

Our results support this proposed mechanism. First, the absence of a real change in secondary structure content upon lipid binding agrees with the concept that bundle opening occurs without helical disruption. Second, our experimental determination of the orientation of apoE3(1–183) $\alpha$-helices in disc complexes agrees with such a model. Even the short helix connecting H1 and H2 seems to adopt the predicted orientation. Third, the following geometric considerations are consistent with this model. The disc particles employed in the present study have a molecular weight of 468,000 and a protein:lipid
molar ratio of ~200:1. If each disc particle contained three apoE3(1–183) and 600 DMPC, the calculated molecular weight of the complex would be 470,000, in good agreement with the value determined by flotation equilibrium analysis. In our model, when the bundle opens with helices 1 and 2 on one side and helices 3 and 4 on the other, we estimate that the elongated structure is about 116 Å long (see Fig. 4). Assuming a helix diameter of 15–17 Å (15), two helices oriented side by side are large enough to effectively cover the DMPC bilayer thickness. Thus, three elongated, open molecules could cover 348 Å, in agreement with a disc circumference of 345 Å, as derived from gradient gel electrophoresis. In the case of larger diameter discs, it is plausible that additional apoE3(1–183) could be accommodated, adopting a similar orientation.

Recently, De Pauw et al. (15) described the organization of apoE helices in DMPC disc complexes wherein antiparallel 17-residue helices surround the disc, oriented parallel with respect to the lipid chains. Such a model, however, is hard to reconcile with the length of helical segments present in the lipid free globular conformation of the N-terminal domain (helices 3 and 4 comprise 37 and 32 amino acids, respectively). Indeed, in this case a disruption of the helical segments found in the lipid-free globular conformation must be imposed. This is likely to be an energetically unfavorable reorientation and, in the case of helix 4, cannot explain the profound effect of the Arg-Cys interchange (apoE3 and apoE2 isoforms) at position 158 in terms of receptor binding ability (30). Moreover, using the same geometrical arguments described above, their model is not in agreement with the disc diameter.

The recently reported x-ray crystal structure of a human apolipoprotein A-I N-terminal deletion mutant (Δ1–44) apoA-I provides additional support for the model presented in Fig. 4. Borbani et al. (31) showed that this protein adopts an elongated, horseshoe-shaped conformation. This structure is predicted to resemble the lipid-associated conformation of apoA-I (32). Furthermore, the authors suggest that apoA-I may interact with disc particles and spherical high density lipoproteins, forming a “belt” that surrounds the lipid complex. In the case of discs, it is envisaged that two apoA-I molecules could circumscribe the periphery of the disc aligning perpendicular to the phospholipid fatty acyl chains with retention of specific helical pairings observed in the antiparallel dimer seen in the crystal structure of this protein. Although our data are consistent with a simple conformational opening of the apoE3(1–183) helix bundle, they do not exclude a belt configuration wherein paired, extended apoE3(1–183) molecules circumscribe the disc perimeter, as suggested for apoA-I discs (31).

The model described here represents a first step toward understanding the interactions between apoE3-lipid particles with lipoprotein receptors. Keeping in mind that lipid association is a requirement for high affinity binding to the LDL receptor (8, 14), the conformational change occurring in the N-terminal, receptor binding domain of apoE3 upon interaction with lipid surfaces is of primary importance. In the absence of modification of the helical structure, we propose that the conformational change is due to a realignment of discrete helical segments, especially the hydrophilic face of helix 4, which contains the amino acids thought to interact with the receptor (amino acids 130–150). Side-chain reorientation could conceivably be attributed to the curvature imposed upon the helices by the disc shape of the particle and/or to the reorientation of the helices with respect to each other when the bundle “opens” and associates with lipid. This rearrangement could modify some of the numerous salt bridges present in the apoE3 N-terminal domain helix bundle conformation (11). It is noteworthy that the recently determined x-ray crystal structure of the ligand binding repeat 5 of the LDL receptor (33) reveals a concave face that could act as an apoE-lipid particle binding surface. Improved knowledge of the receptor-activating conformation of apoE3(1–183) should allow for characterization of the molecular features that modulate receptor-ligand interactions critical to maintenance of plasma lipid homeostasis.

Acknowledgments—We thank Ferry d’Obrenan for preparation of Fig. 4. Grant support from the Alberta Heart and Stroke Foundation is gratefully acknowledged. We also acknowledge Action de Recherches Concertées-Belgium for its financial support. We thank Dr. C.M. Kay for assistance with the analytical ultracentrifugation experiments.

REFERENCES

1. Dergunov, A. D., and Rosseneu, M. (1994) Biol. Chem. Hoppe-Seyler 375, 485–495
2. Zhang, S. H., Reddick, R. L., Fiodrathia, J. A., and Maeda, N. (1992) Science 258, 468–471
3. Plum, A. S., Smith, J. D., Hayek, T., Aalto-Setala, K., Walsh, A., Verstayft, J. G., Rubin, E. M., and Breslow, J. L. (1992) Cell 71, 343–353
4. Strittmatter, W. J., Weisgraber, K. H., Huang, D. Y., Dong, L.-M., Salvesen, G. S., Pericak-Vance, M. A., Schmechel, D. E., Saunders, A. M., Goldgarber, D., and Roses, A. D. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 8098–8102
5. Schmechel, D. E., Saunders, A. M., Strittmatter, W. J., Crain, B. J., Huiette, C. M., Joo, S. H., Pericak-Vance, M. A., Goldgarber, D., and Roses, A. D. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 9649–9655
6. Weisgraber, K. H., and Mahley, R. W. (1996) FASEB J. 10, 1485–1494
7. Wettereau, J. R., Aggerbeck, L. P., Ball, S. C., Jr., and Weisgraber, K. H. (1988) J. Biol. Chem. 263, 6240–6248
8. Innerarity, T. L., Friedlander, E. J., Ball, S. C., Jr., Weisgraber, K. H., and Mahley, R. W. (1988) J. Biol. Chem. 258, 12341–12347
9. Weisgraber, K. H. (1994) Adv. Prot. Chem. 45, 249–302
10. Aggerbeck, L. P., Wettereau, J. R., Weisgraber, K. H., Wu, C.-S., and Lindgren, F. T. (1988) J. Biol. Chem. 263, 6249–6258
11. Wilson, C., Wardell, M. R., Weisgraber, K. H., Mahley, R. W., and Agard, D. A. (1991) Science 252, 1817–1822
12. Breiter, D. R., Kanost, M. R., Benning, M. M., Wesenberg, G., Law, J. H., Wells, M. A., Raymond, I., and Holden, H. M. (1991) Biochemistry 30, 603–608
13. Wang, J., Gagné, S. M., Sykes, B. D., and Ryan, R. O. (1997) J. Biol. Chem. 272, 17912–17920
14. Innerarity, T. L., Pitas, R. E., and Mahley, R. W. (1979) J. Biol. Chem. 254, 4186–4190
15. De Pauw, M., Vanlou, B., Weisgraber, K. H., and Rosseneu, M. (1995) Biochemistry 34, 10953–10960
16. Fisher, C. A., Wang, J., Francis, G. A., Sykes, B. D., Kay, C. M., and Ryan, R. O. (1997) Biochem. Cell Biol. 75, 45–53
17. Jonas, A. (1986) Methods Enzymol. 128, 553–582
18. Nichols, A. V., Krauss, R. M., and Muslinger, T. A. (1986) Methods Enzymol. 128, 417–431
19. Ryan, R. O., Howe, A., and Serab, D. G. (1990) J. Lipid Res. 31, 871–879
20. Nelson, C. A., Lee, J. A., Brewster, M., and Morris, M. D. (1974) Anal. Biochem. 60, 69–74
21. Goormaghtigh, E., de Jongh, H. H. J., and Ruysschaert, J.-M. (1996) Appl. Spectrosc. 50, 1519–1527
22. Goormaghtigh, E., Cabiaux, V., and Ruysschaert, J.-M. (1996) Eur. J. Biochem. 239, 409–420
23. Goormaghtigh, E., and Ruysschaert, J.-M. (1996) in Molecular Description of Biological Membrane Components by Computer Aided Conformational Analysis (Brasseur, R., ed) pp. 285–329, CRC Press, Boca Raton, FL
24. Goormaghtigh, E., Cabiaux, V., and Ruysschaert, J.-M. (1994) Subcell. Biochem. 23, 329–363
25. Frings, U. P., and Gunthard, H. H. (1981) in Membrane Spectroscopy (Grell,
ApoE Conformation in Disc Particles

E., ed) pp. 270–332, Springer-Verlag, Berlin

26. Goormaghtigh, E., Cabiaux, V., and Ruysschaert, J.-M. (1994) Subcell. Biochem. 23, 405–450
27. Kabsch, W., and Sander, C. (1983) Biopolymers 22, 2577–2637
28. Raussens, V., Narayanaswami, V., Goormaghtigh, E., Ryan, R. O., and Ruysschaert, J.-M. (1995) J. Biol. Chem. 270, 12542–12547
29. Wientzek, M., Ray, C. M., Oikawa, K., and Ryan, R. O. (1994) J. Biol. Chem. 269, 4605–4612
30. Dong, L.-M., Parkin, S., Trakhanov, S. D., Rupp, B., Simmons, T., Arnold, K. S., Newhouse, Y. M., Innerarity, T. L., and Weisgraber, K. H. (1996) Nat. Struct. Biol. 3, 718–722
31. Borhani, D. W., Rogers, D. P., Engler, J. A., and Brouillette, C. G. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 12291–12296
32. Rogers, D. P., Brouillette, C. G., Engler, J. A., Tendian, S. W., Roberts, L., Mishra, V. K., Anantharamaiah, G. M., Lund-Katz, S., Phillips, M. C., and Ray, M. J. (1997) Biochemistry 36, 288–300
33. Fass, D., Blacklow, S., Kim, P. S., and Berger, J. M. (1997) Nature 388, 691–693