Lipid binding-induced conformational changes in the N-terminal domain of human apolipoprotein E

Carl A. Fisher and Robert O. Ryan[1]

Lipid and Lipoprotein Research Group, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2S2

Abstract **The N-terminal domain of human apolipoprotein E3 (apoE3) adopts an elongated, globular four helix bundle conformation in the lipid-free state. Upon lipid binding, the protein is thought to undergo a significant conformational change that is essential for manifestation of its low density lipoprotein receptor recognition properties. We have used fluorescence resonance energy transfer (FRET) to characterize helix repositioning which accompanies lipid interaction of this protein. ApoE3(1–183) possesses a single cysteine at position 112 and four tryptophan residues (positions 20, 26, 34, and 39). Modification of Cys112 with the chromophore, N-iodoacetyl-N′-(5-sulfo-1-naphthyl)etheylenediamine (AEDANS) was specific and did not alter the secondary structure content of the protein. The efficiency of energy transfer from donor Trp residues to the AEDANS moiety was 49% in buffer, consistent with close proximity of the chromophores. Guanidine HCl titration experiments induced characteristic changes in the efficiency of energy transfer, indicating that FRET data faithfully reports on the conformational status of the protein. Interaction of AEDANS-apoE3(1–183) with dimyristoylphosphatidylcholine to form disk particles, or with detergent micelles, resulted in large decreases in the efficiency of energy transfer. Distance calculations based on the FRET measurements revealed that lipid binding increases the average distance between the four Trp donors and the AEDANS acceptor from 23 Å to 44 Å. The results obtained demonstrate the utility of FRET to investigate conformational adaptations of exchangeable apolipoproteins and are consistent with the hypothesis that, upon lipid binding, apoE3(1–183) undergoes conformational opening, repositioning helix 1 and 3 to adopt a receptor-active conformation.—**Fisher, C. A., and R. O. Ryan. **Lipid binding-induced conformational changes in the N-terminal domain of human apolipoprotein E.** ***J. Lipid Res.*** **1999.** 40: **93–99.**

Supplementary key words fluorescence • AEDANS • tryptophan • energy transfer • lipid binding • apolipoprotein • conformation

Human apolipoprotein E (apoE) is a 34 kDa, 299 amino acid exchangeable apolipoprotein which is a key component of triacylglycerol-rich lipoproteins. ApoE, which exists as one of three predominant isoforms, E2, E3 or E4 (1), has been shown to function in lipoprotein metabolism, Alzheimer's disease, and nerve regeneration (2, 3). There is extensive evidence that apoE plays a key role in facilitating clearance of lipoprotein particles from plasma via its ability to bind cell surface receptors including the low density lipoprotein (LDL) receptor (2) and the LDL receptor-related protein (4).

Structural studies of apoE (5, 6) revealed it is comprised of two independently folded protein domains that can be isolated after treatment of the protein with thrombin. The 22 kDa N-terminal domain (residues 1–191) adopts a water-soluble, monomeric globular conformation that is resistant to denaturation. By contrast, the 10 kDa C-terminal domain (residues 216–299) is less stable to denaturation, has a high lipid binding affinity, and is responsible for self association of apoE in the absence of lipid. In terms of binding to the LDL receptor, several lines of evidence have led to a consensus which localizes the receptor binding region of apoE to the N-terminal domain, specifically between residues 130 and 150 (1). This region of the protein is rich in basic amino acids and their proposed role in receptor interactions is consistent with studies demonstrating loss of receptor binding after chemical modification of lysine and arginine residues (7, 8). In the absence of lipid, the isolated N-terminal domain is not recognized by the LDL receptor. However, complexation with phospholipids to form discoidal structures results in a particle that binds efficiently to the LDL receptor (9). This behavior is similar to that observed for full-length apoE, which binds to LDL receptors on fibroblasts only after complexation with lipid (10). These data support the view that a lipid binding-induced conformational adaptation of apoE, which can be effectively mimicked by the isolated N-terminal domain, is an essential feature of apoE function as a ligand for receptor-mediated endocytosis of plasma lipoproteins.

Abbreviations: apo, apolipoprotein; AEDANS, N-iodoacetyl-N′-(5-sulfo-1-naphthyl)ethylenediamine; DMPC, dimyristoylphosphatidylcholine; FRET, fluorescence resonance energy transfer; SDS, sodium dodecyl sulfate; TFE, trifluoroethanol.

[1]To whom correspondence should be addressed.

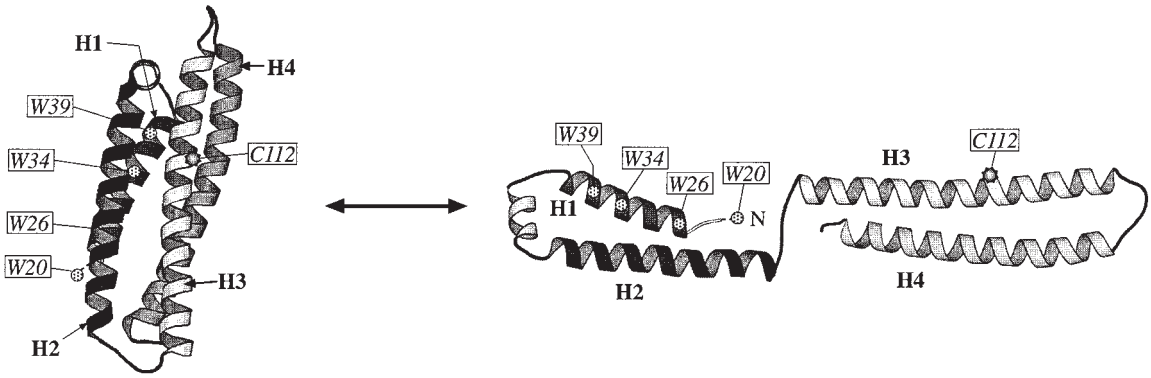

Fig. 1. Model depicting a lipid association-induced conformational change of apoE3 N-terminal domain. The ribbon structure on the left corresponds to the globular lipid-free four helix bundle, established by X-ray crystallography (11). Conformational opening of the bundle is depicted on the right, with helices 1 and 2 moving away from helix 3 and 4 about a putative hinge region located in the loop between helix 2 and helix 3. Note that the helical boundaries present in the bundle conformation are maintained in the 'open' conformation. T he position of Trp and Cys residues in the protein are noted. Adapted from Weisgraber (1).

In 1991, Wilson et al. (11) reported the X-ray crystal structure of the 22 kDa N-terminal domain of human apoE3 in the lipid-free state. This structure, which is comprised of a bundle of four elongated amphipathic α-helices, bears a remarkable similarity in molecular architecture to full length apolipophorin III (apoLp-III) from *Locusta migratoria* (12) and *Manduca sexta* (13). Structural information on these proteins in the absence of lipid has led to the concept that reversible lipid binding is accompanied by a significant conformational change. A model depicting helix bundle apolipoprotein conformational opening is presented in **Fig. 1**. In this model, proposed by Weisgraber (1), the N-terminal domain exposes its hydrophobic interior by 'opening' via a putative hinge region located in the loop between helix 2 and helix 3. Inherent in this model is the prediction that helix 1 and 2 move away from helix 3 and 4 as the bundle 'opens'. Evidence in support of a major conformational change upon lipid binding has been obtained from surface area measurements at the air–water interface which indicate the protein occupies more area per amino acid than can be accounted for if the protein retained its globular structure (14). In the present study we have used fluorescence resonance energy transfer (FRET; see ref. 15 for a review) to characterize helical repositioning that accompanies lipid binding of this protein domain and compared the data obtained with that predicted by the open conformation model.

MATERIALS AND METHODS

Protein expression and AEDANS labeling

Recombinant apoE3(1–183) was expressed and purified as described by Fisher et al. (16). Isolated protein was stored lyophilized at −20°C. Five hundred nmoles protein was incubated in 50 mm Tris-HCl, pH 8.0, with 0.1 mm dithiothreitol and 500 nmol N-iodoacetyl-N′-(5-sulfo-1-naphthyl)etheylenediamine (AEDANS; Molecular Probes, Eugene, OR) at 37°C, 2.5 h in the dark. Unreacted AEDANS was separated from labeled protein by passage through a Sepharose CL-6B (Pharmacia) column followed by lyophilization.

Incorporation of extrinsic fluorophore was confirmed by electro-spray mass spectrometry and/or absorption spectroscopy. That is:

$$\frac{A_{336}}{\varepsilon} \times \frac{\text{MW protein}}{[\text{protein}]} = \frac{\text{moles fluorophore}}{\text{moles protein}}$$

where A is the absorbance and ϵ is the molar extinction coefficient of the fluorophore. The labeled apoE sample displayed characteristic fluorescence properties of AEDANS, including an excitation maximum of 336 nm and an emission maximum of 478 nm.

Fluorescence measurements

For FRET experiments, protein samples were excited at 295 nm and emission spectra were recorded from 300 nm to 575 nm on a Perkin-Elmer Luminescence Spectrometer LS50, 3–8 nm slit widths for both excitation and emission. Wild type and AEDANS-apoE3(1–183) samples were dissolved in 50 mm sodium phosphate, pH 7.0. All spectra were recorded at 20°C. Prior to measurement, samples were allowed to equilibrate to holder temperature for 10 min. Blank spectra were generated from identical solutions lacking protein and were subtracted from spectral data. Corrections for dilutions were made where necessary. Where indicated, AEDANS–apoE3(1–183) was incubated with the appropriate concentration of guanidine HCl, trifluoroethanol (TFE), or detergent for 30 min at room temperature prior to analyses. Dimyristoylphosphatidylcholine (DMPC)-apoE3(1–183) complexes were formed as described previously (16).

Intramolecular distance calculations

The efficiency (E) of energy transfer was calculated as follows:

$$E = 1 - \frac{Q_{DA}}{Q_D}$$

where Q_D and Q_{DA} is the quantum yield of the donor in the absence and presence of acceptor, respectively.

The distance between energy donor and acceptor (R) is

$$R = R_o \left(\frac{1}{E} - 1\right)^{\frac{1}{6}}$$

where R_0 is the distance at which the transfer efficiency is 50%,

$$R_o = 9.765 \times 10^3 \, (k^2 J Q_D \, n^{-4})^{\frac{1}{6}}$$

and k^2 is the orientation factor of the donor and acceptor. In the present case the generally accepted value of 2/3 was used on the basis of the assumption of random rotation of the fluorophores (17) and n is the refractive index of the medium between donor and acceptor, and was taken to be 1.4 (18). J is the spectral overlap integral

$$J = \frac{\Sigma \, I_D \, \varepsilon_4 \, \lambda^4 \Delta\lambda}{\Sigma \, I_D \, \Delta\lambda}$$

where I_D is the fluorescence intensity of the donor, ε_A is the molar extinction coefficient of the acceptor, and λ is the wavelength in cm.

Other methods

Circular dichroism spectroscopy, performed as described elsewhere (19), was used to monitor protein stability in buffer and during guanidine HCl titration experiments. Electrospray mass spectrometry was performed as described by Fisher et al. (16).

RESULTS

AEDANS labeling

Recombinant apoE3(1–183) was labeled with AEDANS on Cys112 as described in Materials and Methods. After removal of unbound chromophore, the labeled protein was subjected to electro-spray mass spectrometry. The spectrum revealed a single mass peak corresponding to apoE3(1–183) plus 1 AEDANS unit with no evidence for incorporation of more than one AEDANS per molecule. Using absorption spectroscopy, the extent of labeling was determined to be between 85 and 95%. Circular dichroism spectroscopy revealed that the labeled protein retains its secondary structure content.

Distance measurements in the lipid-free state

Fluorescence emission spectra of unlabeled recombinant apoE3(1–183) and AEDANS–apoE3(1–183) in buffer (excitation 295 nm) are shown in **Fig. 2**. The unlabeled protein sample gave rise to a single emission peak centered around 343 nm, as previously reported by Aggerbeck et al. (5). In the case of AEDANS–apoE3(1–183), the Trp fluorescence emission peak was reduced in intensity and a second peak, of approximately equal intensity, was observed around 480 nm. This corresponds with the known emission properties of AEDANS, whose absorbance spectrum overlaps directly with the emission spectrum of Trp. The appearance of an emission peak at 480 nm, together with the large decrease in Trp emission at 343 nm, suggests that the AEDANS moiety has been excited by energy transfer from Trp residues in helix 1. The magnitude of the spectral changes observed upon AEDANS labeling suggests efficient energy transfer from the donor Trp residues to AEDANS in the helix bundle conformation. As the relative intensity of the AEDANS emission peak was unaffected by dilution, it can be concluded that intermolecular energy transfer does not contribute to the results obtained. Calculations based on the observed energy transfer between Trp and AEDANS moieties in the protein are reported in **Table 1** and yield an average distance between the donor Trp residues and the acceptor AEDANS moiety of 23 ± 2 Å.

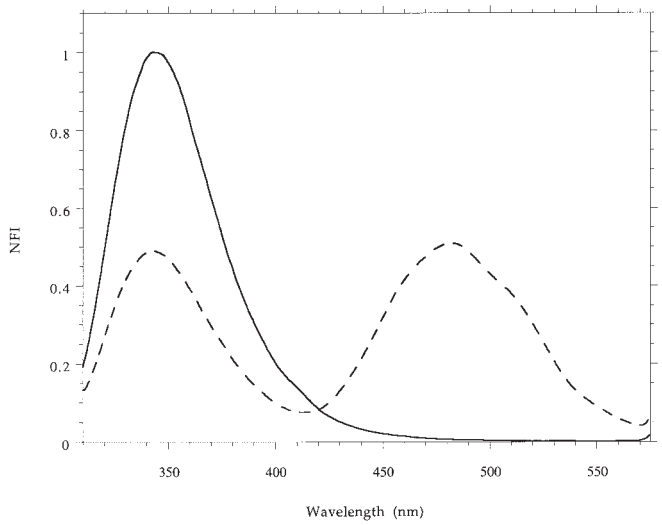

Fig. 2. Fluorescence emission spectra of apoE3(1–183) in buffer. (——), apoE3(1–183); (– – –), AEDANS–apoE3(1–183). One μm protein in 50 mm sodium phosphate, pH 7.0, 20°C was excited at 295 nm. NFI; normalized fluorescence intensity.

Guanidine HCl titration of AEDANS–apoE3(1–183)

To evaluate whether AEDANS labeling affected protein stability, the effect of increasing guanidine HCl concentrations on energy transfer from Trp to AEDANS was investigated (**Fig. 3**). At guanidine HCl concentrations up to 2 m, there was a relatively small decrease in the intensity of the 480 nm emission peak and a corresponding small increase in the 340 nm emission peak. These data suggest that, in 2 m guanidine HCl, apoE3(1–183) retains its globular protein fold, consistent with the findings of others (6). At 2.5 m guanidine HCl and higher, however, a significant decrease in the intensity of the AEDANS emission peak was observed. Likewise, there is a corresponding increase, although less dramatic, in the relative intensity of the Trp emission peak (340 nm). In terms of the Trp fluorescence emission peak, at higher guanidine HCl concentrations, there is a red shift in the emission maximum, indicating that the Trp residues are becoming more exposed to solvent as a function of increasing guanidine HCl concentration. Furthermore, the observation that Trp fluorescence emission intensity is highest at 2.5 m indicates that

TABLE 1. Parameters for resonance energy transfer in lipid-free and lipid-bound AEDANS labeled apoE3(1–183)

	ApoE3(1–183)	ApoE3(1–183)–DMPC
Quantum yield, Q_D	0.20	0.23
Energy transfer efficiency, E (%)	49	2.7
Overlap integral, J^a (cm^6/mol)	6.3×10^{-15}	1.0×10^{-14}
Distance at 50% efficiency, R_o (Å)	22	24

Values are the average of three independent experiments. Quantum yield is based on a value of 0.20 for 30 mm tryptophan in butanol.
[a] Determined as described in Materials and Methods.

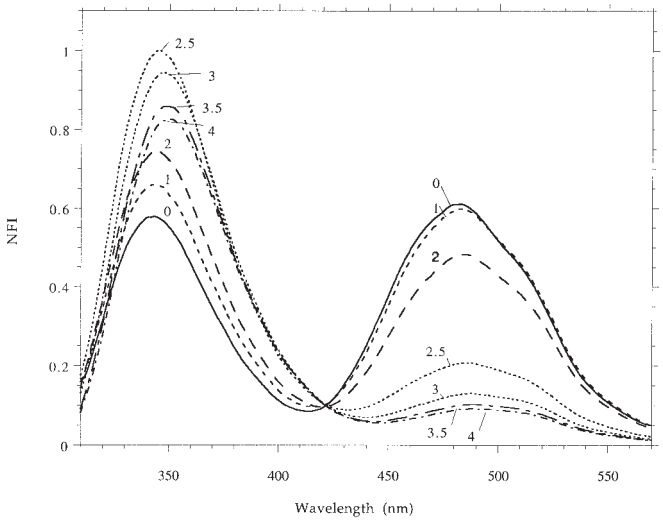

Fig. 3. Effect of guanidine HCl on the efficiency of energy transfer from Trp to AEDANS in AEDANS–apoE3(1–183). Fluorescence emission spectra (excitation 295 nm) obtained from solutions containing 1 μm AEDANS–apoE3(1–183) in 50 mm sodium phosphate, pH 7.0. Samples were pre-incubated in the presence of the indicated molarity of guanidine HCl for 30 min prior to analysis. Spectra were recorded at 20°C.

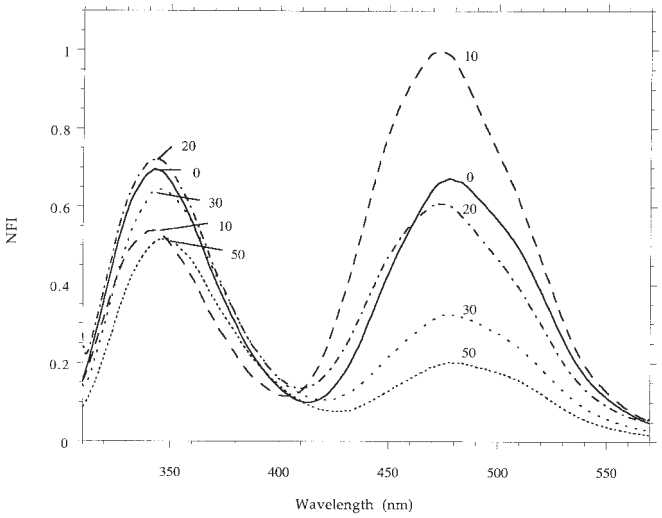

Fig. 4. Effect of trifluoroethanol on energy transfer from Trp to AEDANS in AEDANS–apoE3(1–183). Emission spectra (excitation 295 nm) were obtained on a 1-μm sample of AEDANS–apoE3(1–183) in the absence and presence of the indicated percentages of trifluoroethanol (v/v) in 50 mm sodium phosphate, pH 7.0, 20°C.

quenching of Trp fluorescence occurs at higher guanidine HCl concentrations. A similar quenching behavior was also evident with unlabeled protein upon titration with guanidine HCl (data not shown).

The large decrease in energy transfer observed between 2 and 2.5 m guanidine HCl fits well with denaturation data on the N-terminal domain of apoE3 reported by others (6) and thus provides support for the conclusion that AEDANS modification did not significantly alter the stability properties of the protein. These data also provide validation for the concept that the efficiency of energy transfer seen in buffer is due to the folded state of the protein and that changes in structure or conformation are reflected by changes in energy transfer. In parallel studies using urea as the denaturant, similar results were obtained.

Trifluoroethanol titration of AEDANS–apoE3(1–183)

In previous studies of helix bundle exchangeable apolipoprotein conformation (20), we found that inclusion of 50% (v/v) trifluoroethanol (TFE) as co-solvent induced a conformational change similar to that seen upon lipid binding. This effect was hypothesized to be due to the ability of TFE to provide a lipid mimetic environment, interacting with hydrophobic regions of the protein by displacing helix–helix contacts in the bundle conformation. The effect of TFE on the efficiency of energy transfer in AEDANS–apoE3(1–183) is shown in **Fig. 4**. At 10% (v/v) TFE, an apparent increase in energy transfer was observed, compared to buffer alone, as seen by an increase in AEDANS emission together with a corresponding decrease in Trp fluorescence emission intensity. Based on this information alone it might be concluded that, in the presence of 10% TFE, the protein structure 'tightens,' effectively shortening the distance between the chromophores, increasing the efficiency of energy transfer. This structural alteration could conceivably arise from the known ability of TFE to induce α-helical secondary structure in proteins, including apoE3(1–183) (16). However, in 10% TFE, when the AEDANS moiety was excited directly (336 nm), a significant enhancement of the AEDANS emission peak was observed (data not shown). A corresponding enhancement of tryptophan fluorescence emission was not detected in the case of unlabeled apoE3(1–183), which showed a progressive increase in tryptophan fluorescence quenching as a function of TFE concentration. Thus, we conclude that the enhancement in the AEDANS emission peak (excitation 295 nm) observed in 10% TFE may be due to movement of helix 3 (independent of helix 1), positioning the AEDANS moiety toward the hydrophobic interior of the protein. This interpretation is supported by the 6.5 nm blue shift (482–475.5 nm) in AEDANS fluorescence emission maximum in 10% TFE versus buffer alone (excitation 336 nm). At 20% TFE, a decrease in energy transfer can be seen by the decrease in AEDANS fluorescence emission together with an increase in the tryptophan fluorescence emission peak, versus that seen in 10% TFE. At 30% TFE and 50% TFE, a significant decrease in the AEDANS emission peak is observed that is not accompanied by a corresponding increase in Trp fluorescence emission.

Energy transfer in AEDANS–apoE3(1–183)–DMPC discs and detergent micelles

The experiments described above provide evidence that FRET measurements can provide information about the proximal relationship between helix 1 and helix 3 in AEDANS–apoE3(1–183). On the basis of existing models

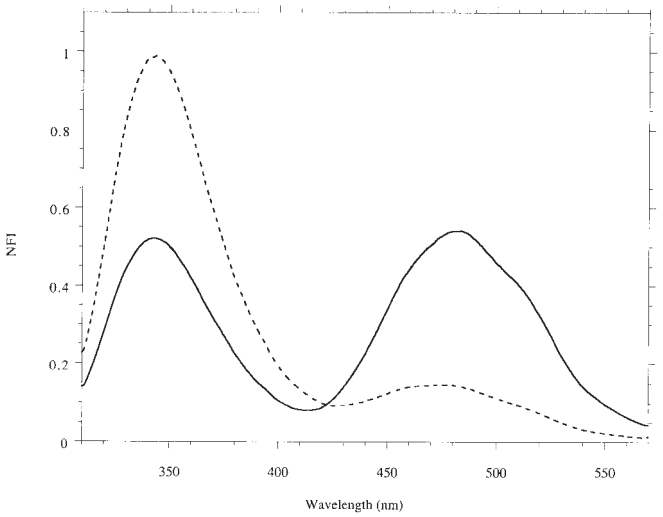

Fig. 5. Effect of interaction with DMPC on the efficiency of energy transfer from Trp to AEDANS in AEDANS–apoE3(1–183). Fluorescence emission spectra (excitation 295 nm) were obtained at 20°C, protein concentration 1 μm; (——), AEDANS–apoE3(1–183) in buffer; (– – –), AEDANS–apoE3(1–183)–DMPC disk complexes in buffer.

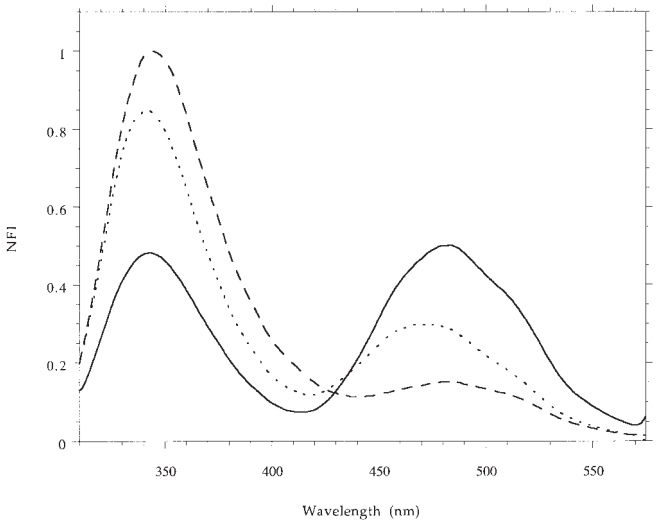

Fig. 6. Effect of detergent micelle interaction on the efficiency of energy transfer in AEDANS–apoE3(1–183). (——), emission spectrum (excitation 295 nm) of 2 μm AEDANS–apoE3(1–183) in 50 mm sodium phosphate, pH 7.0; (– – –), AEDANS–apoE3(1–183) in buffer containing 1% SDS (w/v) and (- - - - - -), AEDANS–apoE3(1–183) in buffer containing 1 mm lysophosphatidylcholine.

of apoE N-terminal domain conformational opening (Fig. 1), it can be predicted that such an opening will result in a decreased efficiency of energy transfer between Trp residues in helix 1 and the AEDANS moiety on helix 3. Comparison of AEDANS–apoE3(1–183) energy transfer efficiency in the lipid-free globular conformation and that when the protein is bound to bilayer disk complexes of DMPC (**Fig. 5** and Table 1) suggests a significant change in the relative positions of the donor and acceptor chromophores. Control spectra of apoE3(1–183)–DMPC disk complexes displayed no fluorescence in the region of AEDANS emission nor was there a significant change in tryptophan fluorescence quantum yield upon binding to DMPC. The average distance between tryptophan residues in helix 1 and the AEDANS moiety in DMPC disks versus that in buffer alone, as well as that predicted on the basis of a simple conformational opening of the helix bundle, are reported in **Table 2**. Given that each disc possesses multiple copies of apoE3(1–183), a portion of the AEDANS fluorescence emission peak in DMPC disc complexes may arise from intra-molecular or inter-molecular energy transfer. To evaluate the extent to which intermolecular energy transfer may contribute to the results seen for lipid associated apoE3(1–183), experiments were performed using detergent micelles as a model lipid surface, under conditions where a maximum of 1 apoE3(1–183) molecule is bound per micelle. The results obtained (**Fig. 6**) showed a similar trend toward less efficient energy transfer when apoE3(1–183) was bound to either SDS or lysophosphatidylcholine micelles.

TABLE 2. Distance measurements derived from FRET analysis of AEDANS labeled apoE3(1–183) in the presence and absence of lipid

	R(Å)	
	Measured[a]	Calculated
ApoE3(1–183)	23 ± 2	21[b]
ApoE3(1–183)–DMPC[c]	44 ± 4	58[d]

[a] From FRET measurements.
[b] Average distance between centers as per PDB co-ordinates of Wilson et al. (11).
[c] DMPC discs were made as per Fisher et al. (16).
[d] Derived from the 'open conformation' in the orientation depicted in Fig. 1; distance calculations were determined using the program InsightII (Biosym, San Diego, CA).

DISCUSSION

ApoE plays a critical role in plasma cholesterol homeostasis, where it functions as a ligand for cell surface lipoprotein receptors. The receptor binding site of apoE has been localized to residues 130–150 in the N-terminal domain of the protein (1). Importantly, the receptor recognition properties of this protein are manifest only in a lipid-associated state. The availablility of detailed structural information in the lipid-free state (11) has allowed for the development of hypotheses about lipid binding-induced conformational changes. In the present study we have used FRET to investigate helix repositioning of the N-terminal domain four-helix bundle upon lipid association, obtaining evidence in favor of a conformational 'opening' of the bundle.

ApoE3(1–183) contains four tryptophan residues (positions 20, 24, 36, 39) and a single cysteine at position 112. From the X-ray structure of this protein it is known that the four tryptophan residues are centered around helix 1 (residues 23 to 42) of the four-helix bundle while Cys112 is located in the C terminal half of helix 3. Tryptophan

residues can function as excellent intrinsic energy donors while Cys112 provides a site for specific attachment of an extrinsic energy acceptor. From the work of others, it is recognized that Cys112 is available for covalent modification (21). In the present study we covalently modified the sulfhydryl group of Cys112 with AEDANS, a chromophore which has been extensively used in FRET studies (22–25). AEDANS serves as a suitable energy acceptor in this system because its absorption spectrum overlaps directly with the emission spectrum of tryptophan. The present system is advantageous because the natural locations of tryptophan and cysteine in the folded protein are known from the crystal structure, providing an important reference standard for FRET studies of lipid binding-induced conformational changes. Indeed, from the X-ray coordinates, the average distance from the compound donor to the acceptor Cys is ~21 Å, in good agreement with the value of 23 Å derived from FRET measurements. Furthermore, the helix bundle conformation is monomeric in solution, which minimizes intermolecular contributions to FRET (5)

Figure 1 depicts a model of a putative 'open conformation' of apoE3 N-terminal domain wherein helix 1 and 2 move away from helix 3 and 4 by rotating about a hinge region located in the loop between helix 2 and 3. Such an opening of the molecule would result in exposure of the hydrophobic faces of the amphipathic α-helices which are otherwise sequestered in the interior of the bundle conformation. These hydrophobic regions are thought to interact with lipid surfaces, creating a stable, yet reversible, binding interaction. An alternate model, presented by de Pauw et al. (26), suggests that interaction with DMPC to form bilayer disc complexes induces the helical segments in apoE to realign, forming antiparallel 17 residue helices which orient parallel to the phospholipid fatty acyl chains. Considering the width of an α-helix to be 15–16 Å, and introducing "breaks" in helix 2, 3, and 4, the estimated average distance between Trp residues in helix 1 and Cys112 would approach 64 Å. While the FRET data reported herein do not exclude this model, this postulated molecular organization is not consistent with recently acquired infrared data on apoE3(1–183)–DMPC disc complexes (27) or the predicted effect that realignment of helix 4 (residues 130–164) into two helices would have on the relative position of Arg150. The orientation of this residue relative to the defined LDL receptor binding region (residues 130–150) has been shown to be an important determinant of marked differences in apoE3/apoE2 isoform specific receptor binding properties (28).

Guanidine HCl titration experiments revealed important points about this system. First, changes in the efficiency of energy transfer correlated well with the known denaturation properties of this protein (6). While the folded structure displayed efficient energy transfer, guanidine HCl-induced denaturation resulted in a dramatic decrease in energy transfer (Fig. 3). Thus, it can be concluded that AEDANS modification of apoE3(1–183) does not alter the stability properties of the protein. Second, from changes in energy transfer observed upon protein unfolding, it is evident that changes in the conformation of apoE3(1–183) will be reflected in FRET measurements. Similar conclusions were drawn from experiments performed using TFE as a co-solvent. Decreased energy transfer observed as a function of TFE concentration are consistent with the hypothesis that, above 20% (v/v), TFE induces conformational opening of the helix bundle through replacement of helix–helix contacts with helix–TFE interactions. In this case hydrophobic contacts between helices in the bundle conformation would be lost, with the protein no longer constrained to retain its globular structure. Studies in the presence of either TFE or guanidine HCl were complicated by the fact that tryptophan fluorescence quenching is observed. For example, the transition from 20% TFE to 30% TFE induced a large decrease in AEDANS emission, whereas this was not accompanied by a corresponding increase in tryptophan fluorescence emission, due to a progressive quenching by TFE of Trp fluorescence. By contrast, the quantum yield of AEDANS fluorescence emission in AEDANS–apoE3(1–183) was unaffected between 20 and 75% TFE (direct excitation at 336 nm) yet increased between 0% TFE and 10% TFE. Thus, distance measurements under these conditions must take into account changes in donor and/or acceptor fluorescence quantum yield, which have a direct effect on energy transfer.

When complexed with DMPC, the fluorescence quantum yield of tryptophan in unlabeled apoE3(1–183) was not significantly altered. However, AEDANS–apoE3(1–183) displayed reduced energy transfer compared to the lipid-free state, resulting in an increase in the measured distance between the compound energy donor and the AEDANS moiety from 23 Å to 44 Å. As the secondary structure content of the protein was not affected by DPMC binding, we conclude that interaction of apoE3(1–183) with lipid surfaces induces a conformational change that increases the distance between helix 1 and helix 3. While these data are consistent with the open conformation model presented in Fig. 1, a simple opening of the helix bundle would be expected to yield a larger distance between donor and acceptor moieties in this protein (Table 2). Further experiments will be required to better define the precise nature of the conformational change and to minimize potential sources of error in the FRET-derived distance calculations. Experimental factors that could contribute error to the observed results include *1*) alterations in the orientation factor, k^2, of the donor or acceptor species in the different conformations; *2*) the prescence of a compound energy donor (e.g., four Trp residues) in the molecule, or *3*) possible effects of the transition of Trp residues from an aqueous medium to a lipid/water interface. With regard to the first point, we have elected to use the averaged value of $k^2 = 2/3$ because, in the present system, the compound energy donor is represented by the sum of the four tryptophan residues. Also, in other systems, anisotropy measurements suggest rapid movement in the AEDANS moiety, suggesting that random orientation may prevail during the energy transfer process (the lifetime of the tryptophan excited state). The problems introduced by the presence of a compound energy donor in this system can most readily be solved by se-

quential site-directed mutagenesis of tryptophan residues in apoE3(1–183) to yield a protein with a single energy donor species. In this case greater accuracy can be achieved in FRET-derived distance measurements. Toward this end, we have recently replaced Trp20 with a tyrosine (W20Y) by site-directed mutagenesis. In buffer, AEDANS-labeled W20YapoE3(1–183) showed an increased energy transfer efficiency compared to corresponding wild-type apoE3(1–183), consistent with the fact that, in the four-helix bundle crystal structure, W20 resides farthest from the site of AEDANS modification (cysteine 112 in helix 3). These data, which illustrate the sensitivity of FRET measurements to alterations in the donor chromophore population in this system, suggest that creation of a single tryptophan mutant apoE3(1–183) will permit highly accurate distance measurements to be obtained. Furthermore, the availability of a tryptophan-deficient apoE3(1–183) can be used to dilute AEDANS-labeled apoE3(1–183) samples, thereby providing an internal control for possible intermolecular energy transfer in the case of disc complexes and spherical lipoprotein recombinants. Finally, further mutations that place a tryptophan residue (or the cysteine residue) on different helices in the bundle will permit detailed characterization of helix repositioning which accompanies lipid interaction and, conceivably, reconstruction of a lipid-bound structure. The general applicability of FRET methods to the class of amphipathic exchangeable apolipoproteins provides a promising approach to study these proteins in their biologically active, lipid-associated state.

The authors thank Dr. C. M. Kay and K. Oikawa for circular dichroism measurements, Dr. Vincent Raussens for assistance with Fig. 1 and Dr. V. Narayanaswami for critical reading of the manuscript and helpful suggestions. CAF is the recipient of a Canadian Heart Foundation Studentship Award and ROR is a Medical Scientist of the Alberta Heritage Foundation for Medical Research. This work was supported by a grant from the Heart and Stroke Foundation of Alberta.

Manuscript received 29 May 1998 and in revised form 10 September 1998.

REFERENCES

1. Weisgraber, K. H. 1994. Apolipoprotein E: structure–function relationships. *Adv. Protein Chem.* **45:** 249–302.
2. Mahley, R. W. 1988. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science.* **240:** 622–630.
3. Weisgraber, K. H., and R. W. Mahley. 1996. Human apolipoprotein E: the Alzheimer's disease connection. *FASEB J.* **10:** 1485–1494.
4. Kowal, R. C., J. Herz, J. L. Goldstein, V. Esser, and M. S. Brown. 1989. Low density lipoprotein receptor-related protein mediates uptake of cholesteryl esters derived from apoprotein E–enriched lipoproteins. *Proc. Natl. Acad. Sci. USA.* **86:** 5810–5814.
5. Aggerbeck, L. P., J. R. Wetterau, K. H. Weisgraber, C-S. C. Wu, and F. T. Lindgren. 1988. Human apolipoprotein E3 in aqueous solution II. Properties of the amino- and caboxyl-terminal domains. *J. Biol. Chem.* **263:** 6249–6258.
6. Wetterau, J. R., L. P. Aggerbeck, S. C. Rall, Jr., and K. H. Weisgraber. 1988. Human apolipoprotein E3 in aqueous solution I. Evidence for two structural domains. *J. Biol.Chem.* **263:** 6240–6248.
7. Mahley, R. W., T. L. Innerarity, R. E. Pitas, K. H. Weisgraber, J. H. Brown, and E. Gross. 1977. Inhibition of lipoprotein binding to cell surface receptors of fibroblasts following selective modification of arginyl residues in arginine-rich and B apoproteins. *J. Biol.Chem.* **252:** 7279–7287.
8. Weisgraber, K. H., T. L. Innerarity, and R. W. Mahley. 1978. Role of lysine residues in plasma lipoproteins in high affinity binding to cell surface receptors on human fibroblasts. *J. Biol. Chem.* **253:** 9053–9062.
9. Innerarity, T. L., B. J. Friedlander, S. C. Rall, Jr., K. H. Weisgraber, and R. W. Mahley.1983. The receptor binding domain of human apolipoprotein E. Binding of apolipoprotein E fragments. *J. Biol. Chem.* **258:** 12341–12347.
10. Innerarity, T. L., R. E. Pitas, and R. W. Mahley. 1979. Binding of arginine rich (E) apolipoprotein after recombination with phospholipid vesicles to low density lipoprotein receptors of fibroblasts. *J. Biol. Chem.* **254:** 4186–4190.
11. Wilson, C., M. R. Wardell, K. H. Weisgraber, R. W. Mahley, and D. A. Agard. 1991. Three dimensional structure of the LDL receptor-binding domain of human apolipoprotein E. *Science.* **252:** 1817–1822.
12. Breiter, D. R., M. R. Kanost, M. M. Benning, G. Wesenberg, J. H. Law, M. A. Wells, I. Rayment, and H. M. Holden. 1991. Molecular structure of an apolipoprotein determined at 2.5 Å resolution. *Biochemistry.* **30:** 603–608.
13. Wang, J., S. Gagne, B. D. Sykes, and R. O. Ryan. 1997. Insight into lipid surface recognition and reversible conformational adaptations of an exchangeable apolipoprotein by multidimensional heteronuclear NMR techniques. *J. Biol. Chem.* **272:** 17912–17920.
14. Weisgraber, K. H., S. Lund-Katz, and M. C. Phillips. 1992. Apolipoprotein E: stucture–function correlations. *In* High Density Lipoproteins and Atherosclerosis. III. N. E. Miller and A. R. Tall, editors. Elsevier, Amsterdam. 175–181.
15. Selvin, P. R. 1995. Fluorescence resonance energy transfer. *Methods Enzymol.* **246:** 300–334.
16. Fisher, C. A., J. Wang, B. D. Sykes, C. M. Kay, G. A. Francis, and R. O. Ryan. 1997. Bacterial expression, isotope enrichment, and NMR analysis of the N-terminal domain of human apolipoprotein E. *Biochem. Cell Biol.* **75:** 45–53.
17. Chapman, E. R., K. Alexander, T. Vorherr, E. Carafoli, and D. R. Storm. 1992. Fluorescence energy transfer analysis of calmodulin–peptide complexes. *Biochemistry.* **31:** 12819–12825.
18. Lakowicz, J. R. 1983. Principles of Fluorescence Spectroscopy. Plenum Press, New York.
19. Ryan, R. O., K. Oikawa, and C. M. Kay. 1993. Conformational, thermodynamic and stability properties of *Manduca sexta* apolipophorin III. *J. Biol. Chem.* **268:** 1525–1530.
20. Narayanaswami, V., C. M. Kay, K. Oikawa, and R. O. Ryan. 1994. Structural and binding characteristics of the carboxyl terminal fragment of apolipophorin III from *Manduca sexta. Biochemistry.* **33:** 13312–13320.
21. Weisgraber, K. H. 1990. Apolipoprotein E distribution among human plasma lipoproteins: role of cysteine–arginine interchange at position 112. *J. Lipid Res.* **31:** 1503–1511.
22. Lakey, J. H., D. Baty, and F. Pattus. 1991. Fluorescence energy transfer distance measurements using site-directed single cysteine mutants: the membrane insertion of Colicin A. *J. Mol. Biol.* **218:** 639–653.
23. Lakey, J. H., D. Duché, J-M. González-Mañas, D. Baty, and F. Pattus. 1993. Fluorescence energy transfer distance measurements. The hydrophobic helical hairpin of colicin A in the membrane-bound state. *J. Mol. Biol.* **230:** 1055–1067.
24. Steer, B. A., and A. R. Merrill. 1994. The colicin E1 insertion-competent state: detection of structural changes using fluorescence resonance energy transfer. *Biochemistry.* **33:** 1108–1115.
25. Ebu Isaac, V., L. Patel, T. Curran, and C. Abate-Shen. 1995. Use of fluorescence energy transfer to estimate intramolecular distances in the Msx-1 homeodomain. *Biochemistry.* **34:** 15276–15281.
26. de Pauw, M., B. Vanloo, K. H. Weisgraber, and M. Rosseneu. 1995. Comparison of lipid binding and lecithin:cholesterol acyltransferase activation of the amino- and carboxyl-terminal domain of human apolipoprotein E3. *Biochemistry.* **34:** 10953–10960.
27. Raussens, V., C. A. Fisher, E. Goormaghtigh, R. O. Ryan, and J-M. Ruysschaert. 1998. The low density lipoprotein receptor active conformation of apolipoprotein E. Helix organization in N-terminal domain-phospholipid disc particles. *J. Biol. Chem.* **273:** 25825–25830.
28. Dong, L-M., S. Parkin, S. D. Trahkanov, B. Rupp, T. Simmons, K. S. Arnold, Y. M. Newhouse, T. L. Innerarity, and K. H. Weisgraber. 1996. Novel mechanism for defective receptor binding of apolipoprotein E2 in type III hyperlipoproteinemia. *Nature Struct. Biol.* **3:** 718–722.