Structural Characterization of a Low Density Lipoprotein Receptor-active Apolipoprotein E Peptide, ApoE3-(126–183)*

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Apolipoprotein E (apoE) plays a critical role in lipoprotein particle clearance from blood plasma through its interaction with the low density lipoprotein (LDL) receptor and other related receptors. Here, we studied a 58-residue peptide encompassing the receptor binding region of apoE. ApoE3-(126–183) was generated by cyanogen bromide cleavage of recombinant apoE3-(1–183), purified by reversed-phase high pressure liquid chromatography, and characterized by mass spectrometry. Far UV CD spectroscopy of the peptide showed that it is unstructured in aqueous solution. The addition of trifluoroethanol or dodecylphosphocholine induces the peptide to adopt an α-helical conformation. ApoE3-(126–183) efficiently transforms dimyristoylphosphatidylglycerol (DMPG) vesicles into peptide-lipid complexes. Analysis of apoE3-(126–183)-DMPG complexes by electron microscopy revealed disc-shaped particles with an average diameter of 13 ± 3 nm. Flotation equilibrium analysis yielded a particle molecular mass of 252 kDa. Far UV CD analysis of apoE3-(126–183)-DMPG discs provided evidence that the peptide adopts a helical conformation. Competition binding experiments with 125I-labeled low density lipoprotein (LDL) were conducted to assess the ability of apoE3-(126–183)-DMPG complexes to bind to the LDL receptor. Both N-terminal apoE and the peptide, when complexed with DMPG, competed with 125I-LDL for binding sites on the surface of cultured human skin fibroblasts. Under the conditions employed, apoE3-(126–183)-DMPG complexes were similar to apoE3-(1–183)-DMPG discs in their ability to bind to the receptor, demonstrating that the peptide represents a good model to study the interaction between apoE and the LDL receptor. Preliminary NMR results indicated that a high resolution structure of the apoE3-(126–183) peptide is obtainable.

Human apolipoprotein E (apoE) is a 299-amino acid protein implicated in plasma lipid metabolism, Alzheimer’s disease, and nerve regeneration (1). ApoE plays a key role in lipoprotein particle clearance from the blood plasma through its interaction with members of the low density lipoprotein (LDL) receptor family (2). ApoE exists as one of three predominant isoforms (1). The most abundant isoform (ApoE3) contains cysteine at position 112 and arginine at position 158. ApoE2 has cysteines at both positions 112 and 158, while apoE4 has arginines at these positions. Structural characterization studies (3, 4) have shown that apoE is composed of two independently folded domains. The 10-kDa C-terminal domain (residues 216–299) has a high lipid binding affinity and is responsible for apoE self-association in the absence of lipid. The 22-kDa N-terminal domain (residues 1–191) adopts a water-soluble, monomeric globular conformation that is resistant to denaturation. The N-terminal domain associates poorly with plasma lipoproteins (5, 6), suggesting that either the lipid binding affinity of this domain is weak or the C-terminal domain is required to initiate association with lipoprotein particles.

The LDL receptor binding site of apoE has been localized to the N-terminal domain of the protein between residues 130 and 150 (1). This region 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, apoE does not recognize the LDL receptor, while complexation of full-length apoE or the isolated N-terminal domain with lipid results in particles that bind efficiently to the LDL receptor (9). These data suggest that a lipid binding-induced conformational change in apoE, or more specifically the N-terminal domain, is essential for apoE to serve as a ligand for receptor-mediated endocytosis of plasma lipoproteins.

The x-ray crystal structure of the N-terminal domain in the lipid-free state is known (10). This domain is composed of four extended amphipathic α-helices connected by short loops. The global fold is a helix bundle wherein the nonpolar faces of the α-helices are directed toward the center of the bundle, adopting a leucine zipper-like motif. In addition, numerous salt bridge interactions exist within helices and on adjacent helices, probably contributing to the unusually high stability of this domain compared with other apolipoproteins. The x-ray structure of the N-terminal domain of apoE has led to a model of the lipid-associated conformation of the protein wherein it adopts an open conformation to manifest receptor binding competence (1).

Many synthetic peptides have been used to study the structural and/or functional characteristics of different regions of apoE. Two peptides, apoE-(263–286) and apoE-(267–289), have been studied by CD and NMR in the presence of DSS. Since apoE-(263–286) was shown to bind to DMPC, it was proposed to...
be a primary lipid-binding region of apoE (11, 12). Meredith and co-workers (13) characterized a highly conserved anionic domain of apoE (residues 41–60) and used side chain lactam bridges at different positions to constrain the peptide in different conformations. Their most active peptide consisted of short helices linked by a turn (13). This peptide is able to increase the binding of LDL particles to fibroblast cell surfaces independent of the LDL receptor, possibly through a novel member of the scavenger receptor family, SR-AI (14–16).

Nevertheless, most work with peptide fragments of apoE has been dedicated to the receptor binding region. Since Innerarity et al. (9) showed in 1983 that a peptide isolated following chemical cleavage of apoE and complexed with lipid could bind to the LDL receptor, synthetic peptides have been designed to find the minimal sequence needed that retains this property. In 1985, Sparrow et al. (17) showed that, among other peptides containing the receptor binding region of apoE, apoE3-(126–169) forms complexes with DMPC. Later it was shown that an N,N-diearyl derivative of glycine of the peptide, diC16-GlyapoE3-(126–169), has a high affinity for lipid and a high helical content in the presence of lipid and was capable of enhancing the uptake and degradation of LDL by fibroblasts (18). Another synthetic peptide, a tandem repeat of the amino acids 141–155 inhibited 125I-LDL degradation by human fibroblasts (19, 20). Further modification of this dimeric peptide by acetylation at its N terminus increased its ability to bind cholesterol-rich lipoproteins, enhancing their clearance in vivo (21). Recently, apoE-(130–152) has been studied using two-dimensional homonuclear NMR spectroscopy in the presence of dodecylphosphocholine (DPC) micelles and shown to adopt a helical conformation over its entire length (22). In the absence of data demonstrating that this peptide is able to interact with the LDL receptor, however, it is difficult to know if apoE-(130–152) is in a receptor-active conformation.

In the present study, we have purified a 58-residue peptide, apoE3-(126–183), encompassing the receptor binding region of apoE and demonstrate its ability to adopt a helical conformation in phospholipid disc complexes that bind the LDL receptor. Moreover, two-dimensional heteronuclear NMR data showed that resolution of the structure of this peptide is feasible.

**EXPERIMENTAL PROCEDURES**

**Peptide Purification**—Recombinant apoE3-(1–183), expressed and purified according to Ref. 23, was resuspended at a final concentration of 5 mg/ml in 4 mM guanidine HCl, 0.1 mM HCl. CNBr was added at a CNBr/methionine ratio of >100 and incubated under N2 atmosphere for 24 h in the dark. After quenching the reaction by the addition of excess water, the sample was frozen and lyophilized to remove residual CNBr. The sample was resuspended in water and subjected to reversed-phase HPLC using a semipreparative Zorbas RX-C18 column with a water (plus 0.05% trifluoroacetic acid) to acetonitrile (plus 0.05% trifluoroacetic acid) 0.5%/min gradient. Isolated apoE3-(126–183) was lyophilized, dissolved in a minimal volume of 0.1 M HCl, and relyophilized to remove residual trifluoroacetic acid.

**Phospholipid Disc Formation and Purification**—DMPC (Avanti Polar Lipids) was resuspended in CHCl3/CH3OH (3:1 (v/v) ratio) in a glass tube and dried under vacuum (16 h). The dried lipid sample was dispersed in prewarmed (37 °C) buffer (10 mM Tris-HCl, pH 7.2, 150 mM NaCl, 0.5 mM EDTA) to a final lipid concentration of 5 mg/mg and vortexed for 1 min. From this solution, small unilamellar vesicles (~200 nm in diameter) were prepared by extrusion using 200-nm filters (24) with a miniextruder (Avanti Polar Lipids).

ApoE3-(1–183) or apoE3-(126–183) were complexed with DMPC by mixing preformed DMPC vesicles with the corresponding protein (or peptide) resuspended in water, at a DMPC/protein weight ratio of 2.5:1 and then incubating overnight at 24 °C with gentle shaking. The sample was adjusted to a density of 1.10 g/ml with KBr (final volume 2.5 ml), transferred to a 5-ml quick-seal ultracentrifuge tube, overlaid with 0.9% saline, and centrifuged at 416,000 × g for 3 h at 4 °C. Fractions (300 μl) were collected from the top and assayed for protein using the bicinchoninic acid protein assay (Pierce). Fractions containing protein were pooled and dialyzed against 20 mM sodium phosphate buffer (pH 7.2) and stored at 4 °C.

**Light Scattering Spectroscopy**—A PerkinElmer Life Sciences spectrophotometer (model LS-50B) was used to monitor DMPC vesicle clearance as a result of association with apolipoprotein (or peptide) by 90° light scattering (25). Excitation and emission wavelengths were set to 580 nm with a slit width of 3 nm. The temperature inside the cuvette was regulated at 32 °C, and all solutions were preincubated at this temperature. Buffer (10 mM Tris-HCl, pH 7.2, 150 mM NaCl, 0.5 mM EDTA) was added to DMPC vesicles (350 μg) to a final volume of 1 ml and incubated for 10 min. Specified apolipoproteins or peptides were then added and rapidly mixed, and the change in light scattering was monitored as a function of time.

**CD Spectroscopy**—Far UV CD spectroscopy was performed on a Jasco J-720 spectropolarimeter (Jasco Inc.) as described previously (26).

**Hydrodynamic Studies**—Flotation equilibrium experiments were carried out at 20 °C in a Beckman XLI analytic ultracentrifuge using interference optics following procedures described by Nelson et al. (27) to experimentally determine both the partial specific volume (v) and the apparent weight average molecular weight (Mapp). Three aliquots of apoE3-(126–183)-DMPC complexes were dialyzed against three buffer solutions containing 100 mM Tris (pH 7.2) and different concentrations of KBr or NaBr, providing solvent densities of 1.25, 1.50, and 1.35 g/ml and protein concentrations of 0.50, 0.59, and 0.91 mg/ml, respectively. The solvent densities were calculated using the program SEDNTERP. An aliquot (110 μl) of each sample solution was loaded into a six-sector CFE sample cell along with 115 μl of the corresponding dialysate for each solution. Runs were performed at speeds of 10,000 and 14,000 rpm, and each speed was maintained until there was no significant difference in scans taken 2 h apart to ensure that equilibrium was achieved.

**Electron Microscopy**—Electron microscopy was performed on a Philips EM420 as described previously (28).

**LDL Receptor Binding Competition Assay**—Normal human skin fibroblasts were grown to 60% confluence in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum. Subsequently, cells were switched to medium containing 10% delipidated fetal bovine serum. At confluence, cells were cooled on ice for 30 min, washed twice with phosphate-buffered saline containing fatty acid-free albumin, and incubated for 2 h at 4 °C in Dulbecco’s modified Eagle’s medium, 1 mg/ml fatty acid-free albumin, 1.5 μg/ml 125I-labeled LDL (specific activity ~300 cpn/ng of protein), and 20 μg of receptor-binding competitor. The medium was removed, and the cells were washed twice with phosphate-buffered saline/fatty acid-free albumin and twice with phosphate-buffered saline. The cells were then dissolved by incubation with 0.1 M NaOH at 4 °C for 24 h. The relative ability of DMPC-apolipoprotein complexes to compete for LDL receptor binding sites was determined by measuring cell-associated radioactivity in a Beckman LS 6000 TA liquid scintillation spectrometer. Cell protein was determined by the bicinchoninic acid protein assay using bovine serum albumin as standard. LDL was isolated from human plasma by sequential density centrifugation (29) and was iodinated according to Langer et al. (30). ApoE-(126–183) was enriched in 15N by culturing bacteria harboring the apoE3-(1–183)/pET plasmid construct in M9 minimal medium containing 15NCl as the sole nitrogen source (23) and purified as described above. A sensitivity-enhanced 1H-15N heteronuclear single quantum correlation (HSQC) spectrum (31) was acquired with the peptide resuspended in 50% TFE-d6, 40% H2O, 10% D2O, pH 3.3, at a final concentration of ~2 mM. The HSQC spectrum was acquired at 30 °C on a Varian Unity INOVA 500 spectrometer with the following parameters: 128 and 896 complex points, respectively, for 15N and 1H dimension. The spectral widths were 1115 Hz in 15N and 6000 Hz in 1H dimension. The carrier positions were at 115.3 and 4.75 ppm, respectively, in 15N and 1H dimension.

**RESULTS**

**Purification of the Peptide**—ApoE3-(126–183) was obtained from recombinant human N-terminal apoE3 (residues 1–183) following CNBr chemical cleavage (see “Experimental Procedures”). The peptide was purified by reversed-phase HPLC (Fig. 1) and further characterized by mass spectrometry (Fig. 1, inset). According to the HPLC trace and mass spectrometry data, the peptide is ~99% pure with a mass of 6595.1 ± 1.2 Da, in agreement with the expected theoretical average calculated mass (6594.5 Da) of the peptide. After HPLC purification, the peptide was lyophilized and stored at -20 °C until use.
Characterization of ApoE3-(126–183)—The CD spectrum of apoE3-(126–183) in water (pH 3.3) revealed a random coil, nonstructured conformation (Fig. 2, solid line). Above pH ∼4, the peptide tends to aggregate (data not shown). Upon the stepwise addition of TFE (0–50%, v/v), the helical content of the peptide increases up to 69%, as shown by the increase in negative ellipticity at 222 nm (Fig. 2, and Table I). Likewise, upon the progressive addition of DPC, a micelle-forming detergent, an α-helical conformation (68% in presence of 13 mM DPC) is induced in the peptide (Fig. 2, dotted line, and Table I).

Characterization of the ApoE3-(126–183)-DMPG Complexes—Most exchangeable apolipoproteins possess the capacity to transform phospholipid unilamellar vesicles at the gel-liquid crystalline phase transition temperature into discoidal complexes. During lipid vesicle transformation trials with apoE3-(126–183), we observed that negatively charged DMPG unilamellar vesicles were rapidly cleared at 23 °C, indicating efficient transformation into lipid complexes. Under the same conditions, DMPC vesicles showed a significantly slower clearance rate (data not shown). Thus, we used DMPG vesicles for studies of peptide-lipid interaction, lipid particle production, and characterization. ApoE3-(126–183)-DMPG disc complexes were purified on a density gradient prior to transformation into discoidal particles—

Because interaction of the peptide with DMPG at 23 °C is extremely rapid, we conducted studies at 32 °C. At this temperature, using apoE3-(1–183), the rate of transformation of DMPG vesicles into disc particles is still rapid (Fig. 6), requiring ~30 s to reach 50% maximal clearance (t½). The reaction end point was achieved in about 400–450 s. In the case of apoE3-(126–183), 90% of maximal clearance was reached within the time (~5 s) needed to mix the peptide with the vesicles and start recording on the spectrophotometer. The t½, less than 5 s, and maximal clearance is complete within 180 s. It is important to note that to attain the same maximal clearance level, we used twice the molar amount of peptide compared with apoE3-(1–183). Thus, the lipid/protein molar ratio was 135 in the case of the N-terminal domain and 64 in the case of apoE3-(126–183).

Receptor Binding—The ability of apoE3-(126–183)-DMPG discs to compete with 125I-LDL for binding to the LDL receptor was investigated using cultured human skin fibroblasts (Fig. 7). In the absence of competitor, efficient binding of 125I-LDL occurred. This level of cell-associated radioactivity was normalized to 100%. When incubated in the presence of an excess of unlabeled LDL, a dramatic decrease in cell-associated radioactivity was observed. N-terminal apoE complexes with DMPG competes as well as cold LDL for receptor binding with a 77% decrease in cell-associated radioactivity. These data show that the use of DMPG does not prevent apoE binding to the receptor. In the case of peptide-DMPG complexes, a 73% decrease in 125I-LDL binding was observed, indicating that, under these conditions, apoE3-(126–183) complexed with phospholipids retains 90% (77%/8) of the receptor binding ability of the N-terminal domain.

NMR Study of ApoE3-(126–183)—To test the suitability of the peptide for NMR structure determination, and especially multidimensional NMR experiments, the peptide was enriched with 15N. Recombinant apoE3-(1–183) was expressed in M9 minimal medium containing 15NH4Cl as sole nitrogen source to uniformly 15N label the protein. After CNBr cleavage and purification (see above), ~95% 15N enrichment of apoE3-(126–183) was achieved, as determined by mass spectrometry. A 1H-15N HSQC spectrum of 15N-labeled apoE3-(126–183) was recorded in the presence of 50% TFE (Fig. 8) at pH 3.3. The peaks observed correlate the chemical shift of amide protons with amide nitrogens in the same amino acid. The chemical shifts are well dispersed, resulting in separation of the overall cross-peaks. As expected for a peptide in a α-helical conformation, the central region of the spectrum is crowded and the peaks are generally shifted upfield (32). This spectrum demonstrates that complete assignment of the individual resonances is feasible, permitting calculation of the three-dimensional structure of the peptide.
DISCUSSION

X-ray crystal structures of the N-terminal domain of three natural variants of apoE have been determined, improving our understanding of the structural basis of isoform-specific differences in apoE-mediated lipoprotein metabolism. For example, the E2 isoform possesses dramatically reduced affinity for the LDL receptor. It has been shown that this property arises from an altered salt bridge interaction, which results in relocation of the Arg150 side chain outside of the receptor recognition region (33). The x-ray structure of the N-terminal domain of apoE has also led to a model of the lipid-associated conformation of the protein (1) wherein it is proposed to adopt an open conformation that manifests receptor binding competence. According to this model, the N-terminal domain exposes its hydrophobic interior by opening its structure via a putative hinge region located in the loop between helix 2 and helix 3. Experimental evidence has shown that the N-terminal domain undergoes a conformational change when bound to a lipid surface (34, 35) but does not exclude alternative helix rearrangements. In the absence of a high resolution structure of the lipid-associated form of the protein, the model will remain questionable and is unable to provide a detailed description of the interactions between the protein and the receptor.

Here, taking advantage of the highly efficient system for the expression of the N-terminal apoE3-(1–183) and of the naturally occurring positions of methionine in the protein sequence, we were able to purify a peptide encompassing the receptor binding region of apoE using CNBr cleavage and semiprepara-

| TFE added (%) | α-Helical content | DPC added (mM) | α-Helical content | % |
|---------------|------------------|----------------|------------------|---|
| 0             | 17               | 0              | 17               |   |
| 5             | 19               | 0.28           | 17               |   |
| 10            | 36               | 0.55           | 19               |   |
| 15            | 51               | 0.8            | 23               |   |
| 20            | 60               | 2.8            | 42               |   |
| 25            | 68               | 5.5            | 59               |   |
| 30            | 67               | 8              | 62               |   |
| 50            | 69               | 12.9           | 68               |   |

FIG. 2. Far UV CD spectra of apoE3-(126–183) in water (solid line), in the presence of 13 mM DPC (dotted line), and in the presence of 50% TFE (dashed line). Spectra were recorded at pH 3.3.

FIG. 3. Flotation equilibrium molecular weight determination of apoE3-(126–183)-DMPG complexes at three densities and two rotor speeds, a, b, and c, $r^2$ versus log fringe displacement plots of runs performed at 10,000 (□) and 14,000 rpm (■) and solvent densities of 1.25, 1.30, and 1.35 g/ml, respectively. d, $(1/\omega^2) \times (d \ log \ fringe \ displacement / d \ r^2)$ versus solvent density plot. An average value for the two speed conditions was used for the abscissa. The line was drawn by linear regression to determine the slope ($419 \ RT/\omega^2 M_r$) and y intercept ($r = T/\omega^2$) where $R$ represents the universal gas constant and $T$ represents run temperature in Kelvin.

rally occurring positions of methionine in the protein sequence, we were able to purify a peptide encompassing the receptor binding region of apoE using CNBr cleavage and semiprepara-
micelle-forming detergent, the peptide also adopts an helical propensity do not form helices even at high TFE concentrations (38, 41). In the presence of DPC, a lipid mimetic tends to adopt such a helical conformation (as expected for TFE as a cosolvent). The fact that in the presence of TFE or DPC, we cannot overrule a possible effect of the phospholipid head group on the binding. Segrest et al. (45) predicted that residues 167–182 form an amphipathic α-helix that can bind lipid through its hydrophobic face. The 80% helical content observed for the peptide upon lipid binding is in agreement with this concept. Indeed, the peptide contains the entire fourth helix of the N-terminal apoE bundle (residues 130–164), and, taking into account the predicted helical region between residues 167 and 182, 51 residues should adopt a helical structure in the lipid-associated peptide, representing 87% helical content, in relatively good agreement with the 80% found by CD spectroscopy. This reasoning is also valid for N-terminal apoE3-(1–183) that contains the four helices of the bundle plus the small helix between helix 1 and 2 (i.e. residues 24–42, 44–53, 54–81, 87–122, and 130–164) involving 128 residues. If we add the predicted amphipathic region (residues 167–182), a total of 144 residues should adopt a helical conformation, representing 79% (144/183) of the protein, in good agreement with the CD data. Thus, these results provide evidence that residues 167–182 can form an amphipathic helix in presence of lipid.

Using light scattering, we followed DMPG vesicle transformation upon peptide interaction as a function of time. ApoE3-(126–183) interacts so rapidly that, even working at temperatures above the lipid gel to liquid crystalline phase transition temperature, we could not accurately record a time of half-clearance (t½ < 5 s) of the vesicle sample. Maximal clearance is obtained within the next 180 s. By comparison, apoE3-(1–183) has a t½ of ~30 s, and complete clearance occurs in 400–450 s. The different behavior between the peptide and the N-terminal domain could be tentatively explained by their respective charge density. The overall net charge of apoE3-(1–183) is negative (33 negative and 29 positive charges/183 residues), whereas for apoE3-(126–183) the net charge density is positive (8 negative and 14 positive charges/58 residues). The net positive charge of the peptide could promote rapid binding of
apoE3-(126–183) to the negatively charged DMPG vesicle surface and facilitate transformation of the vesicles into disc particles. If true, this tends to support the concept that transformation of lipid vesicles into disc particles by apolipoprotein is a two-step process and that the binding step is critical in this process (46).

ApoE3-(126–183) peptide fulfills a major requirement of a receptor-active apoE, namely adopting a $\alpha$-helical conformation in a lipid-associated state. Thus, we tested its ability to interact with the LDL receptor. Competition experiments between $^{125}$I-labeled LDL and either N-terminal apoE3-DMPG or apoE3-(126–183)DMPG discs on cultured human skin fibroblasts showed first that N-terminal apoE, when complexed with DMPG, is able to bind to the LDL receptor. This fact is worth noting, since, to the best of our knowledge, receptor binding of apoE bound to a negatively charged phospholipid has not been shown. Second, under the conditions employed, the peptide retains more than 90% of the binding ability seen with apoE3-(1–183).

In 1983, Innerarity et al. (9) showed that a 92-residue CNBr peptide obtained from apoE, apoE3-(126–218), binds to the LDL receptor with an affinity similar to LDL. Here we show that a much shorter 58-residue peptide, apoE3-(126–183), also binds to the LDL receptor. An ability to interact with the LDL receptor could be expected for such a peptide. On one hand, it fulfills major requirements of a receptor-active apoE, as stated above, but also it contains essential regions of apoE known to be important in such an interaction. It contains the putative region of apoE that directly interacts with the receptor (residues 130–150), and it also contains at its C terminus a critical region between residues 170–180. The importance of this region was shown using truncated N-terminal apoE with progressive deletions at the carboxyl terminus (47). These experiments showed that, while apoE3-(1–183) retained nearly full activity, apoE3-(1–174) had only 19% activity, and any further truncation completely abolished receptor binding activity. More recently, point mutation experiments have revealed the importance of this region, especially Arg$^{172}$ (48). It was suggested that association of residues 170–180 with lipid is important either by contributing one or more residues essential for direct interaction with the LDL receptor or by stabilizing or aligning the receptor binding region (residues 130–150) (47, 48).
It should be emphasized that our goal was not to demonstrate that apoE3-(126–183) has the same affinity for the LDL receptor as full-length apoE. Rather, we sought to show that the peptide retains characteristic properties of the N-terminal domain of apoE, including an ability to interact with lipids to adopt a receptor-active conformation. Preliminary NMR results (Fig. 8) indicate the possibility of applying multidimensional NMR for the study of the apoE3-(126–183) peptide, not only in the presence of TFE but also in a lipid mimetic environment such as DPC (data not shown). In the case of DPC, complexation of the peptide with the detergent produces broader peaks in the $^1$H-$^{15}$N HSQC spectrum compared with spectra obtained in the presence of TFE. Line broadening in the presence of lipid is expected and could result in a more difficult assignment process. Nevertheless, the spectra are of much higher quality than that seen for the $^{15}$N-labeled apoE N-terminal domain (23). We are currently pursuing NMR studies of apoE3-(126–183). The structure of the peptide in these two conditions should provide important new insights about the receptor-active conformation of apoE, especially the residues around 170–180, since this region was not “seen” in the crystal structure of the N-terminal apoE bundle (10). It could also clarify the possible role of this region in receptor binding.

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