Lipid-bound Structure of an Apolipoprotein E-derived Peptide*

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Apolipoprotein (apo) E regulates plasma lipid homeostasis through its ability to interact with the low density lipoprotein (LDL) receptor family. Whereas apoE is not a ligand for receptor binding in buffer alone, interaction with lipid confers receptor recognition properties. To investigate the nature of proposed lipid binding-induced conformational changes in apoE, we employed multidimensional heteronuclear NMR spectroscopy to determine the structure of an LDL receptor-active, 58-residue peptide comprising residues 126–183 of apoE in association with the micelle-forming lipid dodecylphosphocholine (DPC). In the presence of 34 mM DPC the peptide forms a continuous amphipathic helix from Glu121 to Arg178. NMR relaxation studies of DPC-bound apoE-(126–183), in contrast to apoE-(126–183) in the presence of TFE, are consistent with an isotropically tumbling peptide giving a global correlation time of ~12.5 ns. These data indicate that the helical peptide is curved and constrained by a lipid micelle consisting of ~48 DPC molecules. Although the peptide behaves as if it were tumbling isotropically, spectral density analysis reveals that residues 150–183 have more motional freedom than residues 134–149. These molecular and dynamic features are discussed further to provide insight into the structural basis for the interaction between apoE and the ligand binding repeats of the LDL receptor.

The fundamental importance of apolipoprotein (apo) E in plasma lipoprotein metabolism is illustrated by transgenic and gene disruption experiments in mice. Transgenic animals over-expressing apoE manifest decreased plasma cholesterol levels and are protected against diet-induced atherosclerosis (1) while apoE-null mice display dramatically elevated plasma lipoprotein and cholesterol levels and are highly susceptible to diet-induced atherosclerosis (2, 3). When complexed to lipoproteins, apoE mediates whole particle uptake and removal from the circulation via members of the LDL receptor family.

Biophysical studies reveal that apoE is comprised of two structural domains, a 22-kDa N-terminal (NT) domain and a 10-kDa C-terminal domain (4, 5). The N- and C-terminal domains are connected by a flexible, unstructured, region encompassing amino acids 191–216 that is susceptible to proteolytic cleavage. Studies conducted with isolated domains reveal that the NT domain contains amino acids responsible for binding to the LDL receptor (6). Several lines of evidence have led to a consensus that localizes the receptor-binding site to residues 136–150 (7). 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 following chemical modification of lysine and arginine residues (8, 9). In the absence of lipid, the isolated NT domain is not recognized by the LDL receptor. On the other hand, complexation with phospholipids results in a particle that binds efficiently to the LDL receptor (6). These data indicate that a lipid binding-induced conformational adaptation of apoE, which can be mimicked by the isolated NT domain, is an essential feature of apoE function as a ligand for receptor-mediated endocytosis of plasma lipoproteins.

X-ray crystallography of lipid-free apoE3-NT has yielded high resolution structures (10, 11). This domain exists as an elongated globular four-helix bundle. Each α-helix segment is amphipathic, orienting its hydrophobic face toward the center of the bundle. The structure of lipid-free apoE3-NT provides a useful starting point for development of models of how the helix bundle alters its structure upon interaction with lipid surfaces to adopt a receptor-active conformation. Weisgraber et al. (12) studied the surface properties of apoE3-NT at the air/water interface on a monolayer balance. These investigators concluded that the protein spreads on the surface to occupy a volume greater than can be accounted for by the globular helix bundle conformation. More recently, NMR spectroscopy studies have provided evidence for a major conformational change in the NT domain upon interaction with lipid (13). In 1994, Weisgraber (7) proposed an “open conformation” model in which the loop segment that connects helix 2 and helix 3 in the bundle functions as a hinge, about which the protein opens to expose a continuous hydrophobic surface. Raussens et al. (14) investigated the structural organization of apoE3-NT in dimyristoylphosphatidylcholine (DMPC) complexes by infrared spectroscopy. These investigators presented a model wherein apoE-NT adopts an open conformation, circumscribing the perimeter of the disc bilayer with its helical axes aligned perpendicular to the fatty acyl chains of DMPC, to adopt a receptor active conformation (6, 15). Support for this model has been
Lipid-bound Structure of ApoE Peptide

obtained from studies employing fluorescence resonance energy transfer to evaluate distance relationships between specific sites in the protein as a function of lipid binding (16, 17). Lu et al. (18) provided evidence for the conformational opening model by demonstrating that apoE-NT dependent transformation of DMPC bilayer vesicles into disc complexes is abolished when helical segments in the bundle conformation are tethered by disulfide bond engineering.

Using an alternate approach Raussens et al. (19) studied a fragment derived from human apoE. A 58-residue peptide encompassing the receptor binding region of apoE was generated by CNBr cleavage of recombinant apoE3-(1–183), purified and characterized. Far UV CD spectroscopy of the peptide showed that it is unstructured in aqueous solution. Importantly, however, apoE3-(126–183) efficiently transforms anionic phospholipid vesicles into LDL receptor competent, peptide-lipid complexes. Analysis of these complexes by electron microscopy revealed disc-shaped particles with an average diameter of 13 ± 3 nm. Subsequently, the structure of this receptor-active apoE peptide was determined by NMR experiments conducted in the presence of the lipid mimetic cosolvent trifluoroethanol (TFE) (20). In 50% TFE, apoE-(126–183) forms a continuous amphipathic α-helix over residues Thr130–Glu179. To extend these findings and investigate the structural organization of apoE-(126–183) in the presence of lipid, we have determined the structure of apoE-(126–183) in complex with the micelle-forming single acyl chain phospholipid dodecylphosphocholine (DPC). Electrostatic and geometric features of the apoE-(126–183) DPC-bound structure suggest that apoE binds to the LDL receptor by interacting with more than one of its ligand binding domains.

The secondary structure of apoE-(126–183) was determined by NMR experiments conducted in the presence of the lipid mimetic cosolvent trifluoroethanol (TFE) (20). In 50% TFE, apoE-(126–183) forms a continuous amphipathic α-helix over residues Thr130–Glu179. To extend these findings and investigate the structural organization of apoE-(126–183) in the presence of lipid, we have determined the structure of apoE-(126–183) in complex with the micelle-forming single acyl chain phospholipid dodecylphosphocholine (DPC). Electrostatic and geometric features of the apoE-(126–183) DPC-bound structure suggest that apoE binds to the LDL receptor by interacting with more than one of its ligand binding domains. The results are discussed in terms of structural determinants responsible for apoE conformational adaptability and binding to the LDL receptor family.

**EXPERIMENTAL PROCEDURES**

**Preparation of ApoE-(126–183)***—Uniformly 15N-labeled apoE3-(126–183) was prepared as previously described (19, 20).

**NMR Spectroscopy***—NMR experiments were performed on ∼2 mM 15N-labeled apoE-(126–183) in the presence of 34 mM DPC-d₃₈ in 500 μL of 10 mM H₂O/90% D₂O, pH 6.0, containing 0.01% (w/v) NaN₃, and 2 mM 2,2-dimethyl-2-silapentane-5-sulfonate as an internal chemical shift reference. NMR experiments were carried out at 25 °C on Varian NOVA 500 MHz and Unity 600 MHz NMR spectrometers. Data were processed using NMRPipe (21) and analyzed using NMRView (22). Complete 1H and 15N spectral assignments of apoE-(126–183) were obtained using gradient-enhanced three-dimensional 15N-edited TOCSY and NOESY (mix times, 75 ms) experiments to identify spin systems and inter-residue connectivities as described by Withrich (23). Confirmation of side-chain assignment was obtained through the use of three-dimensional HNHB and two-dimensional natural abundance 13C- HSQC spectra.

15N T₁, T₂, and heteronuclear NOE relaxation data were recorded at 25 °C on both 500 and 600 MHz spectrometers using the enhanced sensitivity gradient pulse sequences developed by Farrow et al. (24). The T₁ relaxation decay was sampled at 11 time points on each spectrometer: 11.1, 111, 222, 333, 444, 555, 666, 777, 888, 999, and 1110 ms. The T₂ relaxation decay was sampled at different time points: 16.6, 33.2, 49.8, 66.4, 83.0, 99.7, 116.3, 132.9, 149.5, 166.1, and 182.7 ms on the 500 MHz spectrometer and 16.5, 32.1, 49.6, 66.2, 82.7, 99.3, 115.8, 132.4, 148.9, 165.4, and 182.0 ms on the 600 MHz spectrometer. The exponential decay curves for T₁ and T₂ peak intensities were fit using the in-house program Xcrysiv (inhouse written program; www.pence.ulaval.ca/software). ¹H-¹5N NOE values were obtained from the ratio of the peak intensity from proton-saturated and unsaturated spectra. Reduced spectral density mapping was carried out as described in Farrow et al. (25).

**Structure Calculation**—An ensemble of 147 apoE-(126–183) structures was computed from the distance and dihedral angle restraints available (Table I) starting with an extended chain using a simulated annealing protocol (26, 27) in X-PLOr version 3.851 (28). Inter-proton distance restraints were derived from three-dimensional 15N-edited NOESY experiments recorded with a mix of 75 and 120 ms. Distances were calibrated according to Slupsky and Sykes (29). ψ backbone dihedral angles were calculated based on measured 3JHN-H 1 and 2, and heteronuclear NOE relaxation data were recorded at 25 °C on Varian NOVA 500 MHz and Unity 600 MHz NMR spectrometers. The statistical values for the 50 lowest energy structures are presented in Table I. Families of structures were extracted from the ensemble of structures using the program NMRClust (33). For clustering, the backbone heavy atoms of residues 134–149 of the 50 structures were superimposed, and clustering was based on residues 133–177.

**RESULTS**

**Preparation and Characterization of ApoE-(126–183)**—As previously shown, apoE-(126–183) is insoluble in aqueous solution above pH 4, and below pH 4, the peptide is not structured in water. However, in the presence of either lipids, such as dimyristoylphosphatidylglycerol (DMPC), DPC, or lipid-mimicking agents, such as TFE, it adopts a high (70–80%) helical content (19, 20). Previously, it was determined that 13 mM DPC is sufficient to induce full structuring of this peptide (19). For NMR experiments, we prepared the sample by re-suspending lyophilized 15N-labeled apoE-(126–183) in D₂O/H₂O (9:1, v/v). Owing to residual HCl following lyophilization (19), the pH of the solution was around 3.5. To this solution, in a stepwise manner, deuterated DPC was added from a concentrated stock solution in D₂O. Upon each addition, one-dimensional ¹H and two-dimensional ¹H-¹5N HSQC NMR spectra were recorded. Addition of DPC was stopped when the spectra did not show a change between two consecutive additions (data not shown). The final DPC concentration was 34 mM. Following DPC titration, the pH of the sample was adjusted to 6.0 by stepwise addition of 500 mM NaOD in D₂O.

**Structure of ApoE-(126–183)**—Fig. 1 displays a two-dimensional ¹H-¹5N HSQC NMR spectrum of DPC-bound apoE-(126–183) obtained at 600 MHz. Despite the presence of broader peaks than those observed in 50% TFE (20), most resonances were well resolved. Three pairs of resonances overlap (Glu132 with Arg172, Leu148 with Leu159, and Asp153 with Arg167) and other resonances partially overlap (Ala135 with Leu155 and Arg178, and Arg142 with Asp151, and Gln163). In addition, three residues, including the two N-terminal residues, Leu129, Gln172 and the residue preceding the C-terminal proline, Gly182, are missing.

The secondary structure of apoE-(126–183) was determined

**TABLE I**

| NOE restraint | Energy | Dihedral restraint |
|--------------|--------|--------------------|
| Total, 435   | E_total 69.6 ± 1.4 | E_dihedral 0.3 ± 0.1 |
| Intrasidue, 164 | E_bond 15.2 ± 0.6 | |
| Sequential, 185 | E_angle 47.1 ± 1.2 | |
| Medium range, 86 | E_dist 0.14 ± 0.24 | |

a All structures satisfy experimental restraints with no distance violations greater than 0.2 Å and no dihedral violations greater than 2°.

b Energies for the 50 lowest energy structures, in kcal mol⁻¹.

(2 > = |1 – j| < 4).
using NMR spectroscopy based upon NOE connectivities, the 
H$_{\alpha}$/N$_{\alpha}$ MNR chemical shift index (CSI) (34, 35) and the ratio of 
d$_{\alpha}$/d$_{\beta}$ NOEs (32). A summary of these data is illustrated in 
Fig. 2. Helical secondary structure was defined as previously 
described (20). Fig. 2 shows that DPC-bound apoE-(126–183) is 
composed of a single $\alpha$-helix spanning the sequence from Glu$^{131}$ 
to Glu$^{179}$, with the first five and last four residues unstructured. 
Fig. 2 also shows evidence of the simultaneous presence 
of unambiguous $\delta_{\alpha}$ and $\delta_{\beta}$ NOEs for residues 
Leu$^{133}$–Val$^{135}$, Ala$^{152}$–Asp$^{154}$, and Gln$^{156}$–Arg$^{158}$. According to 
Wüthrich (23), $\delta_{\alpha}$ is characteristic of an $\alpha$-helix whereas 
$\delta_{\beta}$ is characteristic of a 3$^10$-helix. The simultaneous presence 
of both NOEs for the same residue may reflect a degree of 
internal flexibility for these residues.

An amide proton secondary shift plot (Fig. 3) shows a periodicity of 3–4 residues from Thr$^{130}$ to Arg$^{178}$, typical of a curved 
amphipathic $\alpha$-helix (36, 37). In general, hydrophobic residues 
tend to have a different field deviation for this type of structure. 
ApoE-(126–183) deviates somewhat from the ideal situation. 
For example Arg$^{134}$, Arg$^{145}$, and Gln$^{156}$ show unexpected 
field deviations characteristic of hydrophobic residues, while 
Val$^{135}$, Ala$^{160}$, and Val$^{162}$ show upfield deviations. In a helical 
wheel representation of this region of the peptide (data not 
shown), the average structure, resulting from superposition of 
the 50 lowest energy structures, is a long curved helix 
$\alpha$-helix whereas Ala$^{160}$ is located near the polar-nonpolar interface, 
and Ala$^{162}$ is located on the polar face of the helix and on the predicted convex side of the 
curvature, explaining the unexpected upfield deviation observed 
for this hydrophobic amino acid, as the amide proton 
secondary deviation is related to the hydrogen bond length. Two regions of the peptide, around Ala$^{152}$–Asp$^{154}$ and Gln$^{156}$–Gly$^{169}$, show a 3–4 residue periodicity but with a lesser intensity 
and a non-significant amide shift deviation. These regions 
might indicate differences in helix curvature around these 
amino acids since secondary structural data indicate these 
residues are helical. Indeed, previously it was shown with 
model peptides that a lack of amide proton secondary shift 
3–4-residue periodicity is not due to the absence of a helical 
conformation but, rather, comes from a less curved structure 
(37).

The three-dimensional structure of apoE-(126–183) was 
calculated from NOE and dihedral restraints as described under 
"Experimental Procedures." An ensemble of 147 structures was 
computed, none of which contained distance restraint viola- 
tions greater than 0.2 Å or dihedral angle violations greater than 2°. The 50 structures with the lowest calculated total 
energy were selected for further consideration. According to 
PROCHECK-NMR (38), 99.7% of the non-glycine residues have 
($\phi$, $\psi$) angles in the most favored or the additionally allowed 
regions of the Ramachandran plot for these 50 structures (data 
not shown). The average structure, resulting from superposition 
of the 50 lowest energy structures, is a long curved helix 
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backbone RMSD of 0.55 ± 0.11 Å and a heavy atom RMSD of 1.4 ± 0.11 Å (Fig. 4a). As may be observed, while the entire helix remains intact, the relative position of residues 150–178 with respect to residues 134–149 changes. Because of this, we tried to extract conformationally related structure subfamilies using the program NMRCLUST (33). For clustering, the backbone heavy atoms of residues 134–149 of the 50 structures were superimposed, and clustering was based on residues 133–177. Nine subfamilies were obtained with four structures considered as outliers. Among the subfamilies, the first three contain more than 50% of the structures. Fig. 4b shows the representative structures of the three most populated subfamilies in complex with a modeled DPC micelle composed of 54 DPC molecules (40). While the DPC micelle is only a model and is slightly bigger than what we would expect based on the overall correlation time, the complex gives a conceptual idea of how this peptide may adopt different conformations when bound to DPC. It suggests that the C-terminal portion of the peptide (residues 150–183) has a lower affinity for the micelle than residues 134–149. As will be discussed later, this has important implications for the interaction of apoE-(126–183) with the receptor. The structures in each subfamily are well defined over the entire helical length, with RMSDs about the mean coordinate positions of 2.45 Å for subfamily 1, 2.04 Å for subfamily 2, and 1.94 Å for subfamily 3 for backbone atoms of residues 137–173. However, for the most stable helical region of the peptide (residues 134–149, see below), the RMSDs about the mean coordinate positions for backbone atoms are as follows: 0.73 Å for subfamily 1, 0.71 Å for subfamily 2, and 0.66 Å for subfamily 3. This shows that the structure is well defined locally. As observed previously in TFE, the structure subfamilies display differing degrees of helix curvature.

Relaxation Measurements of ApoE-(126–183)—To gain insight into the motions of DPC-bound apoE-(126–183), longitudinal ($T_1$) and transverse ($T_2$) $^{15}$N NMR relaxation times as well as $^1$H-$^{15}$N heteronuclear NOEs were measured. The $R_1$...
The time scales of motions present may be represented by the calculation of the reduced spectral density functions. Fig. 6 illustrates the reduced spectral density functions $J(0)$, $J(\omega_0)$, and $J(0.8\omega_0)$ that describe the motion of the H–N bonds derived from the relaxation parameters $R_1$, $R_2$, and the $^{1}H-^{15}N$ NOE. While the spectral density function at zero frequency, $J(0)$, is sensitive to motions on all time scales, the high frequency spectral density functions $J(\omega_0)$ and $J(0.8\omega_0)$ are sensitive to fast internal motions on the time scales $1/\omega_0$ and $1/0.8\omega_0$ (44).

Fig. 5 illustrates the relative inflexibility of residues 134–149.
These residues exhibit a $J(0.87\text{ps/\text{rad}})$ consistently lower than 7.5 ps/\text{rad}, which indicates a lack of internal flexibility. Not surprisingly, residues 126–130 and residue 182 exhibit $J(0.87\text{ps/\text{rad}})$ spectral densities greater than 15 ps/\text{rad} indicating marked flexibility. Interestingly, residues 150–180 exhibit $J(0.87\text{ps/\text{rad}})$ spectral densities higher than for residues 134–149. At 500 MHz, these spectral densities are higher than 7.5 ps/\text{rad}. It appears that residues 150–154 experience slightly elevated $J(0.87\text{ps/\text{rad}})$, which decreases for residues 156–160. Between residues 164 and 180, $J(0.87\text{ps/\text{rad}})$ increases even more. $J(0)$ data follows the opposite trend for both 500 and 600 MHz data. Residues 134–149 exhibit $J(0)$ values of between 4.6 and 5.3 ps/\text{rad}. Residues 150–162 exhibit $J(0)$ values between 5.0 and 5.2. Finally, between residues 164 and 180, $J(0)$ values are between 3.5 and 5.2.

**DISCUSSION**

ApoE-(126–183) is a peptide that includes a lipid binding region and the LDL receptor binding motif of apoE. We have previously demonstrated that this 58-residue peptide conserves some major functions of the NT domain of apoE (19). An earlier study of the DPC bound structure of a 23-residue peptide, apoE-(130–152), revealed a nearly linear, completely helical conformation that displayed some flexibility (45). Whereas our results agree with the helical character of this region of apoE, we observe a pronounced curvature that, as discussed below, may have functional consequences. Furthermore, although apoE-(130–152) includes residues 136–150, it does not contain other residues known to be required for proper apoE-LDL receptor recognition. For instance, it is known that residues 171–183 are critical for receptor binding, as illustrated by studies of truncated variants of apoE. Whereas apoE-(1–183) has nearly full receptor binding activity apoE-(1–174) has only 19% activity with further truncation reducing this activity to 1% (46). Recently, Arg172 was found to play a key role in receptor binding (47). We previously demonstrated that, when complexed with phospholipid, apoE-(126–183) binds LDL receptors on the surface of human skin fibroblasts (19). Since apoE-(126–183) contains all the residues necessary for full receptor binding, it provides a useful working model for studies designed to elucidate the LDL receptor active conformation of apoE. Extending this concept, we determined the structure of apoE-(126–183) in association with DPC, a zwitterionicsingle acyl chain phospholipid that possesses a polar head group identical to that of phosphatidylcholine (PC). Unlike PC, however, DPC forms micelles whose size is amenable to high resolution NMR (41). NMR relaxation measurements on DPC have shown that it mimics a PC bilayer environment (48).

**Comparison of ApoE-(126–183) in DPC and TFE—ApoE-(126–183) bound to DPC forms a curved amphipathic helix from residue Glu131 to Arg178, in agreement with the structure of this peptide in 50% TFE (20). As with the TFE structure, the helix content of the DPC-bound peptide determined by NMR (83%) is higher than that calculated on the basis of far UV CD spectroscopy (70%). While the secondary structure content of apoE-(126–183) is similar, differences exist with respect to the dynamic behavior of the molecule in TFE versus DPC. In TFE, apoE-(126–183) exhibits more rigidity in the region between residues 149 and 159. Outside this region, residues 131–143 and 163–176 display a plasticity of motion such that the further away from the central region, the greater the movement. This type of dynamic behavior is consistent with a helix tumbling in solution.

When bound to DPC, however, apoE-(126–183) behaves more isotropically as might be expected for a peptide bound to the surface of a micelle. $R_g$, $R_b$, and $^3$H-\text{H} NOE have characteristics that, at first glance, appear to follow conventional relaxation behavior for a globular protein. Residues 134–149 comprise a more rigid region while residues 150–179 constitute a more flexible segment of the polypeptide. Between positions 134 and 149 there are six residues with aliphatic side chains (1 Val and 5 Leu). At residue 151, a series of aspartic acid residues (Asp151, Asp153, and Asp154) appear to disrupt binding of apoE-(126–183) to the micelle. Between residues 159 and 161, the peptide is a bit less flexible yet increases dramatically in elasticity after Tyr162, presumably due to the presence of polar and acidic residues, including Glu168 and Glu171. Flexibility in this region may facilitate receptor interaction by permitting structural adaptation to constraints imposed by individual lipid binding repeats. PhD secondary structure prediction (49) analysis of apoE-(126–183) yields results that are in agreement with the present NMR structure. PhD predicts two helical segments (Thr136–Glu168 and Arg172–Arg180). In the first predicted helix there is a small decrease in the reliability index for residues Leu148–Arg150. This region and the predicted helix interruption from residues 169 to 170, generally coincide with the present NMR-derived flexibility results.

Curvature and flexibility in this peptide likely facilitate interaction with lipid surfaces such as lipoproteins or phospholipid disc complexes. Consistent with these structural characteristics, intact apoE is known to bind different sized lipoprotein particles, from small HDL to large chylomicron remnants. Similar physical properties have also been observed for other apolipoproteins. For example, NMR structures of apoC-I and apoC-II in a lipid-mimicking environment revealed curved helical regions and/or linker regions between helices.
with a loosely defined conformation that confers a flexible curvature to the structures (50, 51). The X-ray structure of an N-terminal deletion mutant of apoA-I, (Δ1–43)apoA-I, is comprised of a series of curved amphipathic helices (52) that, conceivably, could circumscribe the perimeter of an HDL particle. These examples suggest that the features observed for DPC-bound apoE-(126–183) may represent typical characteristics of exchangeable apolipoproteins.

Potential Implications of the ApoE-(126–183) Structure on ApoE-LDL Receptor Interactions—The structure of DPC-bound apoE-(126–183) has implications with respect to apoE ability to bind to the LDL receptor. For example, Lys143 and Lys147 are known to display increased solvent accessibility in the presence of lipid, thereby increasing their electrostatic interaction potential with elements of the LDL receptor (13). This increase in solvent accessibility appears to be a consequence of helix curvature in this region induced by the presence of lipid. Likewise, structuring of residues 165–179 in the presence of lipid most likely facilitates interaction with the LDL receptor since it is known Arg172 is required for optimal binding (47). In the case of DPC micelles it is evident that residues 134–149 are anchored at the lipid interface while the region between residues 165 and 179 appears to bind less tightly. It is conceivable that the smaller size and higher radius of curvature of DPC micelles compared with natural lipoproteins affects the ability of apoE-(126–183) to retain tight contact with the lipid surface. Although residues 165–179 are not as tightly associated with the micelle surface, they are nonetheless helical, suggesting induction of helix in this region of the molecule may be a cooperative event triggered and/or maintained by lipid interaction of another region of the peptide (e.g. residues 134–149).

Structural characteristics of the LDL receptor have been discussed in a preceding study (20). Here, we briefly outline features that are pertinent to this discussion. The presence of highly conserved acidic residues within ligand binding modules of the receptor has led to the hypothesis that ligand-receptor recognition may be due to electrostatic interactions. Although ligand binding repeat 5 (LR5) was demonstrated to be the most important (53), experiments also suggest a role for other repeats, located upstream of LR5 (53, 54). The well-characterized receptor binding region of apoE (136–150) displays a highly positive electrostatic surface potential and thus, may interact with LR5, the most negatively charged ligand binding repeat. Residues 165–179 become structured in the presence of lipid and present another positively charged surface that could interact with a second ligand binding repeat. Glutamic acid residues punctuating the largely positive surface might be important for proper orientation of the peptide at the surface of the receptor, interacting with the few arginine and lysine residues present in almost every ligand binding repeat. Once a ligand-binding repeat, presumably LR5, has recognized the most basic region of apoE (residues 136–150), adjacent repeat(s) might interact with another region(s) of apoE (notably around Arg172). This interaction may orient the apolipoprotein and, by extension, the entire lipoprotein particle, in a favorable manner with respect to the receptor. When bound to DPC or in TFE (20), apoE-(126–183) adopts an elongated structure ~70 Å in length. By the same token, a single ligand-binding repeat has a maximum diameter of 25 Å. These distances are consistent with the possibility that apoE interacts with more than one repeat at the surface of the receptor in a process that would be facilitated by the structural independence of adjoining ligand binding repeats (55, 56). A recently reported crystal structure of the entire extracytosolic domain of the human LDL receptor provides support for this view (57). It is known that, after lipoprotein internalization, at endosomal pH (pH < 6), the LDL receptor discharges its ligand before recycling to the cell surface. Because this crystal structure was obtained at pH 5.3, it should represent the endosomal conformation of the LDL receptor. The most remarkable feature of this structure is that ligand binding repeats LR4 and LR5 are held in place through interactions with the β-propeller motif of the receptor, while other repeats seem to be fixed mainly by intermolecular crystal contacts. The authors suggest that, at endosomal pH, following release of the lipoprotein ligand, the β-propeller can serve as an alternative substrate for the two ligand binding repeats, and this might be a pH-regulated, reversible process. These conclusions favor a predominant role for LR4 and LR5 in ligand binding activity of the receptor, in agreement with concepts presented here and elsewhere (20).
Potential Molecular Mechanism Involved in Structuration of Residues 165–178—The lipid bound structure of apoE-(126–183) raises questions about why helix 4 does not terminate at the same residue in the presence and absence of lipid? In the crystal structure of apoE3-NT domain, helix 4 ends at Ala164 (10). The amino acids around this residue, Val-Tyr-Gln-Ala164-Gly-Ala correspond to a typical Schellman C-capping terminal motif sequence (Fig. 7), commonly found at the end of α-helices (Ref. 58; for a review see Ref. 59). Furthermore, analysis of the x-ray coordinates of apoE3-NT (PDB code: ILPE; 10) provides evidence for the presence of Schellman motif structural requirements (Fig. 7, panel A). Vadar analysis2 revealed the possibility of an H-bond between the NH group of Gly165 and the CO group of Tyr162 (Fig. 7C) corresponding to the C-Cα bond in a Schellman motif. Although a C-Cα H-bond was not detected, Ala166 (corresponding to Cα and the last residue observed in the electron density), has a very high B factor suggesting its position and orientation may not be accurately determined. The angle value for the Gly165 (corresponding to Cα) is 133°, a positive value, as expected for a left-handed amino acid conformation. The last criterion of a Schellman motif, namely a hydrophobic interaction between Cα and Cβ (corresponding to Ala166 and Val161), is more difficult to identify. We used the CSU program (61) to identify potential hydrophobic contacts in the vicinity of Ala166 and Val161. Whereas the side chain of Ala166 is not oriented correctly to promote hydrophobic interaction between these residues, CSU analysis detected a potential hydrophobic interaction between the side chains of Val161 and Leu83 (located at the beginning of helix 3; Fig. 7C). At the same time, Leu83 makes hydrophobic contact with Ala166. Thus, we suspect that the hydrophobic interaction required for stabilization of the C capping motif is provided by a long-range interaction with Leu83.

Other interactions seem to exist between the end of helix 4 and (a) residues connecting helices 2 and 3 (the 80s loop; Ref. 11) and (b) residues located at the end of helix 2 and the beginning of helix 3. Tyr162 forms many hydrophobic interactions through its aromatic ring atoms that orient between helices 2 and 3, locking the end of helix 4 in close proximity to the 80s loop (Fig. 7C). Val103 has a potential for hydrophobic contact with the side chain of Ala166 while Thr94 makes a main chain hydrogen bond with Ala164 as detected by Vadar and CSU. This H-bond serves to stabilize the CO group of Ala164 at the terminus of helix 4. Interestingly, most of the residues described here as involved in stabilizing interactions are conserved among different apoE sequences (Fig. 7B). The only non-conserved residue is Thr94. Insofar as Thr94 is substituted only by amino acids having smaller side-chains, these substitutions are not expected to sterically hinder H-bond formation between the backbone NH group at position 83 and Ala164.

The interaction of apoE-NT with lipid is believed to arise by opening the globular 4-helix bundle about a hinge region between helices 2 and 3 (7). It appears that helices 1 and 2 and helices 3 and 4 remain preferentially paired during the first stage of bundle opening (18) with possible subsequent reorganization of helix segments (16–18). If this view is accurate, then the hinge region, implicated in the first stage of the opening, must include the 80s loop. Opening the bundle in this manner would disrupt interactions between 80s loop residues and the end of helix 4. Subsequent separation of helices 3 and 4 would abolish long-range hydrophobic interactions between Leu83 and Val161, which contribute to stabilization of the Schellman C capping motif. It has been observed that, upon disruption of such hydrophobic interactions (by the presence of a cofactor, for example), helix structure can extend despite the presence of a C-capping consensus sequence or a helix-breaking Gly residue (58).

In summary, we suggest that lipid binding-induced alteration of interactions responsible for termination of helix 4 in lipid-free apoE provides a molecular explanation for the apparent transition of residues 165–178 from unstructured to α-helix, thereby conferring LDL receptor recognition properties to the protein. Reorganization of the hinge region is likely to be an early event in apoE NT lipid binding, and one that triggers structure formation within residues 165–178. In addition to its role in receptor recognition, structure formation in this region may be an important adaptation that leads to a favorable orientation of the protein with respect to the lipid surface. This proposal has specific advantages including the following. 1) It accounts for the fact that the region of apoE around Arg172 adopts a helical structure in the presence of DPC while it is unstructured in absence of lipid. 2) It explains, at the molecular level, how this region can transition from one structure to another (or more exactly to the absence of structure); and 3) it explains why the lipid-bound conformation is required for correct interaction between apoE and the LDL receptor. The lipid surface effectively serves as a molecular switch to modify stabilizing interactions (especially hydrophobic interactions) at one end of the helix bundle, inducing a 15-residue conformational change from unstructured to amphipathic α-helix that orients and aligns essential elements of the receptor binding region of this protein.

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REFERENCES
1. Shimano, H., Yamada, N., Katsuki, M., Yamamoto, K., Gotoda, T., Harada, K., Shimada, M., and Yasaki, T. (1992) J. Clin. Invest. 90, 2084–2091
2. Plump, A. S., Smith, J. D., Hayek, T., Aalto-Setala, K., Walsh, A., Verstuyft, J. G., Rubin, E. M., and Breslow, J. L. (1992) Science 258, 1817–1818
3. Plump, A. S., Smith, J. D., Hayek, T., Aalto-Setala, K., Walsh, A., Verstuyft, J. G., Rubin, E. M., and Breslow, J. L. (1992) Science 258, 1817–1818
4. Wetterau, J. P., Wetterau, J. R., Weisgraber, K. H., Wu, C.-S. C., and Lindgren, F. T. (1988) J. Biol. Chem. 263, 6249–6258
5. Weisgraber, K. H., Lund-Katz, S., and Phillips, M. C. (1992) in Lipoproteins and Atherosclerosis III (Miller, N. E., and Tall, A. R., eds) pp. 175–181, Elsevier, Amsterdam
6. Weisgraber, K. H., Lund-Katz, S., and Phillips, M. C. (1992) in Lipoproteins and Atherosclerosis III (Miller, N. E., and Tall, A. R., eds) pp. 175–181, Elsevier, Amsterdam
7. Weisgraber, K. H., Innerarity, T. L., Pitas, R. E., Weisgraber, K. H., Brown, J. H., and Grone, K. (1977) J. Biol. Chem. 252, 7270–7287
8. Weisgraber, K. H., Innerarity, T. L., and Mahley, R. W. (1978) J. Biol. Chem. 253, 9053–9062
9. Wilson, C., Wardell, M. R., Weisgraber, K. H., Mahley, R. W., and Agard, D. A. (1991) Science 252, 1817–1821
10. Weisgraber, K. H., Innerarity, T. L., Pitas, R. E., Weisgraber, K. H., Brown, J. H., and Grone, K. (1991) Science 252, 1817–1821
11. Wetterau, J. R., Aggerbeck, L. P., Rall, S. C. Jr., and Weisgraber, K. H. (1988) 34341–34347
12. Johnson, B. A., and Blevins, R. A. (1994) J. Biomol. NMR 6, 37–46
13. Lund-Katz, S., Zaiou, M., Wehrli, S., Dhanasekaran, P., Baldwin, F., Weisgraber, K. H., and Phillips, M. C. (2000) J. Biol. Chem. 275, 34459–34466
14. Rausens, V., Fisher, C. A., Goormaghtigh, E., Ryan, R. O., and Raysschaert, J.-M. (1998) J. Biol. Chem. 273, 25825–25830
15. Fischer, C. A., Wang, J., Francis, G. A., Sykes, B. D., Kay, C. M., and Ryan, R. O. (1997) Biochem. Cell Biol. 75, 43–50
16. Fischer, C. A., and Ryan, R. O. (1999) J. Lipid Res. 40, 93–99
17. Fisher, C. A., Narayanaswami, V., and Ryan, R. O. (2000) J. Biol. Chem. 275, 33691–33696
18. Lu, B., Morrow, J. A., and Weisgraber, K. H. (2000) J. Biol. Chem. 275, 20775–20781
19. Rausens, V., Mah, M. K. H., Kay, C. M., Sykes, B. D., and Ryan, R. O. (2000) J. Biol. Chem. 275, 38329–38336
20. Rausens, V., Slupsky, C. M., Ryan, R. O., and Sykes, B. D. (2002) J. Biol. Chem. 277, 29172–29180
21. Delaglio, F., Grzesiek, S., Vuister, G. W., Zhu, G., Pfeifer, J., and Bax, A. (1995) J. Biomol. NMR 6, 277–285
22. Johnson, B. A., and Hlevics, B. A. (1994) J. Biomol. NMR 4, 603–614
23. Wuthrich, K. (1986) NMR of Proteins and Nucleic Acids, John Wiley & Sons, New York
24. Farrar, N. A., Muhandiram, R., Singer, A. U., Pasel, S. M., Kay, C. M., Giah,

2 Vadar, www.pence.ualberta.ca/software/vadar.
25. Farrow, N. A., Zhang, O., Szabo, A., Torchia, D. A., and Kay, L. E. (1995) J. Biomol. NMR 6, 153–162
26. Nilges, M., Clore, M., and Gronenborn, A. M. (1988a) FEBS Lett. 229, 317–324
27. Nilges, M., Gronenborn, A. M., Brunger, A. T., and Clore, M., (1988b) Protein Eng. 2, 27–38
28. Brunger, A. T. (1992) X-PLOR, version 3.1. A system for X-ray crystallography and NMR, Yale University Press, New Haven, CT.
29. Slupsky, C. M., and Sykes, B. D. (1995) Biochemistry 34, 15953–15964
30. Bax, A., Vuister, G. W., Grzesiek, S., Delaglio, F., Wang, A. C., Tschudin, R., and Zhu, G. (1994) Methods Enzymol. 239, 79–105
31. Karplus, M. (1963) J. Am. Chem. Soc. 85, 2870–2871
32. Gagne, S., Tsuda, S., Li, M. X., Chandra, M., Smillie, L. B., and Sykes, B. D. (1994) Protein Sci. 3, 1961–1974
33. Kelly, A. L., Gardner, S. P., and Sutcliffe, M. J. (1996) Protein Eng. 9, 1063–1065
34. Wishart, D. S., Sykes, B. D., and Richards, F. M. (1991) J. Mol. Biol. 229, 317–324
35. Wishart, D. S., and Sykes, B. D. (1994) Methods Enzymol. 239, 363–392
36. Kurtz, I. D., Kosen, P. A., and Craig, E. C. (1994) J. Am. Chem. Soc. 113, 1406–1408
37. Zhou, N. E., Zhu, B.-Y., Sykes, B. D., and Hodges, R. S. (1992) J. Am. Chem. Soc. 114, 4330–4336
38. Laskowski, R. A., Rullman, J. A. C., MacArthur, M. W., Kaptein, R., and Thornton, J. M. (1996) J. Biomol. NMR 8, 477–486
39. Kabach, W., and Sander, C. (1983) Biopolymers 22, 2677–2637
40. Tieleman, D. P., van der Spoel, D., and Berendsen, H. J. C. (2000) J. Phys. Chem. B 104, 6380–6388
41. Henry, G. D., and Sykes, B. D. (1994) Methods Enzymol. 239, 515–525
42. Kay, L. E., Torchia, D. A., and Bax, A. (1989) Biochemistry 28, 8972–8979
43. Brutscher, B., Bruschweiler, R., and Ernst, R. R. (1997) Biochemistry 36, 13043–13053
44. Viles, J. H., Donne, D., Kroon, G., Prusiner, S. B., Cohen, F. E., Dyson, H. J., and Wright, P. E. (2001) Biochemistry 40, 2743–2755
45. Clayton, D., Brereton, I. M., Kroon, P. A., and Smith, R. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 12291–12296
46. Russell, D. W., Brown, M. S., and Goldstein, J. L. (1988) J. Biol. Chem. 263, 21682–21688
47. Esser, V., Limbird, L. E., Brown, M. S., Goldstein, J. L., and Russell D. W. (1988) J. Biol. Chem. 263, 13282–13290
48. Beglova, N., North C. L., and Blacklow, S. C. (1999) Biochemistry 38, 3926–3935
49. North, C. L., and Blacklow, S. C. (2001) Biochemistry 40, 2008–2015
50. Rudenko, G., Henry, L., Henderson, K., Ichtehenko, K., Brown, M. S., Goldstein, J. L., and Deisenhofer, J. (2002) Science 298, 2353–2354
51. Schellman, C. (1989) in Protein Folding (Steinbach, R., ed) pp. 53–61, Elsevier/ North Holland, New York
52. Harper, E. T., and Rice, G. D. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 2261–2265
53. Sobolev, V., Sorokine, A., Prilusky, J., Abola, E. E., and Edelman, M. (1999) Bioinformatics 15, 327–332
