The carboxyl terminus in apolipoprotein E2 and the seven amino acid repeat in apolipoprotein E-Leiden: role in receptor-binding activity

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Abstract Both apolipoprotein (apo) E2 and apoE-Leiden (tandem repeat of amino acids 121–127) are associated with type III hyperlipoproteinemia and bind defectively to low density lipoprotein receptors. Removing the carboxyl terminus of both variants (residues 192–299) increases receptor-binding activity, suggesting that the carboxyl terminus modulates activity. To identify the region(s) that modulated binding activity, we produced carboxyl-terminal truncations in apoE2 and apoE-Leiden (terminating at positions 191, 223, 244, and 272) and in apoE3 (terminating at positions 191, 223, and 244) and compared their receptor-binding activities as dimyristoylphosphatidylcholine (DMPC) discs. The results suggest that the entire carboxyl terminus up to residue 272, not a discrete smaller segment, is responsible for the modulation in apoE2. Intact apoE-Leiden and the 223 and 244 variants displayed similar activities (~25% of apoE3's), but the 191 variant's activity was identical to that of intact apoE3. ApoE-Leiden and its truncated variants formed larger DMPC discs than did intact or truncated apoE3 or apoE2. These discs contained more apoE molecules than apoE3 discs, suggesting that the apparently normal binding activity of the apoE-Leiden 191 variant results from an increased number of apoE molecules and that the binding activity is actually defective. Direct comparison in a solid-phase assay revealed that the binding activity of the apoE-Leiden fragment was defective (51.4 ± 9.4%). Thus, the defective binding of apoE-Leiden results from a direct effect of the seven amino acid repeat on receptor-binding activity rather than from an indirect effect operating through the carboxyl terminus as previously believed.—Dong, L-M., T. L. Innerarity, K. S. Arnold, Y. M. Newhouse, and K. H. Weisgraber. **The carboxyl terminus in apolipoprotein E2 and the seven amino acid repeat in apolipoprotein E-Leiden: role in receptor-binding activity. *J. Lipid Res.* 1998.** 39: **1173–1180.**

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Human apolipoprotein (apo) E (299 amino acids) is polymorphic with three common isoforms, designated apoE2 (Cys 112, Cys 158), apoE3 (Cys 112, Arg 158), and apoE4 (Arg 112, Arg 158) (1, 2). Through its interaction with lipoprotein receptors, apoE mediates the clearance of very low density lipoproteins (VLDL) and chylomicron remnants (3, 4). Defective binding of apoE to these receptors results in an accumulation of cholesterol-rich lipoprotein particles in the plasma and is an underlying cause of type III hyperlipoproteinemia, a genetic disorder characterized by elevated plasma cholesterol and triglyceride levels and accelerated coronary artery disease (5). Apolipoprotein E2, which has ~1% of the receptor-binding activity of apoE3 (2), is associated with recessive inheritance of this disorder because homozygosity for this isoform in combination with additional genetic or environmental factors is required for expression (5). Apolipoprotein E-Leiden (a mutant with a tandem repeat of amino acid residues 121–127) (6, 7) and other rare variants of apoE (5) are associated with dominant inheritance because heterozygosity for these variants is sufficient to cause type III hyperlipoproteinemia (5).

Structurally, apoE (299 amino acids) contains two domains: the receptor-binding amino-terminal domain (amino acids 1–191, 22-kDa fragment) and the lipid-binding carboxyl-terminal domain (amino acids 216–299, 10-kDa fragment) (8, 9). The receptor-binding region is located in the vicinity of residues 136–150 (3, 10–12). The defective receptor binding of apoE2 arises from two effects: a major effect resulting from critical salt bridge rearrangements caused by substitution of cysteine for arginine at position 158 and a minor effect resulting from modulation by the carboxyl terminus (13–15).

Abbreviations: apo, apolipoprotein; DEAE, diethylaminoethyl; DMPC, dimyristoylphosphatidylcholine; HPLC, high-performance liquid chromatography; LDL, low density lipoproteins; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; VLDL, very low density lipoproteins.

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Although it is not known how the seven amino acid repeat of apoE-Leiden affects receptor-binding activity, removal of the carboxyl terminus (residues 192–299) increases binding activity from ~25% to ~100% of that of apoE3 (7). Therefore, it was suggested that removal of the carboxyl-terminal domain releases conformational constraint on the receptor-binding region, resulting in increased binding of both apoE2 and apoE-Leiden (7, 14).

To determine whether similar regions in the carboxyl terminus are involved in both apoE variants, we produced a series of carboxyl-terminal truncation variants terminating at positions 191, 223, 244, and 272 in apoE2 and at positions 191, 223, and 244 in apoE-Leiden and compared their receptor-binding activities. The results suggest that the entire carboxyl terminus of apoE2 up to residue 272 is involved in the modulation of the receptor binding. They further suggest that the defective binding of apoE-Leiden is a direct effect of the seven amino acid insertion on the conformation of the receptor-binding region rather than an indirect effect operating through the carboxyl terminus as previously believed.

MATERIALS AND METHODS

Construction and expression of apoE mutants

The glutathione S-transferase fusion expression vector pGEX3X (16) was used for expression of apoE mutants. The carboxyl-terminal truncations of apoE were created by polymerase chain reaction as previously described (17). Construct sequences were verified by double-stranded DNA sequencing (18). Expression plasmids were transformed into the *Escherichia coli* strain DH1 (Invitrogen). Transformants with high expression of the GST-apoE fusion protein were grown to mid-log in LB medium at 37°C. Expression was induced by adding isopropyl β-thiogalactoside (0.01% final concentration) to the culture.

The expressed fusion protein was purified by glutathione-agarose affinity chromatography as previously described (17). The fusion protein was complexed with dimyristoylphosphatidylcholine (DMPC) (Sigma) and then cleaved with Factor Xa (Haematologic Technologies) at a ratio of 200:1 (w:w, fusion protein:Factor Xa) at 0°C overnight. The truncation variants were purified by heparin-Sepharose affinity chromatography followed by diethylaminoethyl (DEAE) ion exchange high-performance liquid chromatography (HPLC) (17). Intact human apoE2, apoE3, and apoE-Leiden were isolated from the $d < 1.006$ g/ml lipoprotein fraction as described previously (19). Intact apoE-Leiden was obtained from a heterozygote for this variant (apoE-Leiden: apoE2, 9:1).

LDL receptor–binding assays

Cell culture assay. Human LDL were isolated from plasma of normal fasting subjects by sequential ultracentrifugation (20) and radiolabeled by the iodine monochloride method (21). The various apoEs were mixed with DMPC at a ratio of 1:3.75 (w:w, protein:DMPC), and phospholipid:protein complexes were isolated by density gradient ultracentrifugation (22). The VLDL-like emulsion particles were prepared as described previously (23–25). Briefly, triolein (100 mg) and egg yolk phosphatidylcholine (25 mg) (Sigma) were mixed and then dried under a stream of nitrogen. After resuspension in 5 ml of 10 mm Tris-Cl buffer (pH 8.0) containing 0.1 m KCl and 1 mm EDTA, the materials were sonicated as previously described (24). The mean size of the emulsion particles prepared by this procedure is 35.8 ± 14.9 nm, in contrast to 39.5 ± 18.7 nm for the native VLDL (25). The emulsion particles were incubated with apoE3 or apoE-Leiden at 37°C for 2 h. Particle-bound apoE was separated from unbound apoE on a Superose 6 column (Pharmacia Fine Chemicals). One week before the experiment, normal human fibroblasts were plated at 3.5×10^4 cells/dish. On day 5, the cells were switched to medium containing 10% lipoprotein-deficient serum. On day 7, the cells were incubated in medium containing 2.0 μg/ml of ^{125}I-labeled LDL and various concentrations of apoE–DMPC. The competitive binding of apoE·DMPC versus ^{125}I-labeled LDL was assayed at 4°C as previously described (22).

Solid-phase assay. Recombinant apoE3, apoE2, and apoE-Leiden 22-kDa fragments were isolated as previously described (15), and receptor-binding activity was determined with a solid-phase assay (26). The fragments (100 ng/well) in phosphate-buffered saline (150 mm NaCl, 20 mm sodium phosphate, pH 7.4) (PBS) were incubated overnight at 4°C in 96-well microtiter plates (Dynatech Immunlon, Chantilly, VA). After each subsequent step, the plates were washed with 1% bovine serum albumin (BSA) in PBS. Nonspecific binding was blocked with 4% BSA in PBS for 1 h at room temperature. The soluble LDL receptor fragment, isolated as described (26), was diluted to approximately 10 ng/ml in 2 mM phosphate and 0.15 m NaCl (pH 7.2) containing 3% BSA and 20 mm $CaCl_2$ and incubated in the 22-kDa apoE–coated wells for 2 h at room temperature. Bound receptor was detected with the anti-LDL receptor monoclonal antibody C7 (Amersham), followed by horseradish peroxidase–labeled anti-mouse immunoglobulin G (IgG) (Amersham) and color development with O-phenylenediamine dihydrochloride (Sigma) according to the manufacturer's instructions. Determinations were performed in triplicate in three separate plates. In parallel wells without added receptor, an anti-apoE antibody was used for detection to ensure that the microtiter wells were coated with comparable amounts of each apoE isoform.

Determination of apoE–DMPC disc size

The DMPC particles were stained on the surface of carbon fluid grids. Electron micrographs were made at a magnification of 200,000× and imported via a video camera into an Image 1/AT image-analysis system. The particle size was analyzed by automated sizing and counting programs available on system software (version 4.03a, Universal Imaging Corporation). Multiple areas on a single grid were sampled. To compare the disc sizes by gel filtration, a Superdex 200 column (10/50 HR, Pharmacia), equilibrated with 20 mm sodium phosphate (pH 7.4) containing 150 mm NaCl, was used. The apoE·DMPC complexes (50 μl) were applied to the column and eluted at rate of 0.5 ml/min. The number of apoE molecules/disc was calculated based on the disc volume and molecular weight of apoE as previously described (27).

Determination of apoE distribution among plasma lipoproteins

Apolipoprotein E was iodinated with the Bolton-Hunter reagent (Dupont NEN) (22); specific activities ranged from 150 to 900 dpm/ng. The iodinated protein was reduced with β-mercaptoethanol (0.1% final concentration) and incubated with normal human plasma at 37°C for 2 h as described previously (17). The plasma was fractionated into various lipoprotein classes by Superose 6 column chromatography (10/50 HR, Pharmacia). The column was eluted with 20 mm phosphate buffer (pH 7.4) containing 150 mm NaCl at a flow rate of 0.5 ml/min, and 0.5-ml fractions were collected. The ^{125}I content was determined in a Beckman 8000 counter (Beckman Instruments).

RESULTS

To identify the region(s) of the apoE2 carboxyl terminus that modulates the receptor-binding amino-terminal domain, we produced in *E. coli* a series of carboxyl-domain apoE2 variants truncated at positions 191, 223, 244, and 272. As controls, a series of apoE3 truncations was also produced. Carboxyl-terminal truncation of apoE3 did not significantly affect receptor-binding activity; all of the truncated variants displayed binding activities similar to that of intact apoE3 (**Fig. 1A**). The concentrations of apoE3 variants required for 50% replacement of ^{125}I-labeled LDL ranged from 0.01 to 0.02 μg/ml, which is at the lower end of the normal range for intact apoE3 (10, 14).

In contrast, significant differences in binding activities were observed for the truncated apoE2 variants (Fig. 1B and **Table 1**). As in previous studies, intact apoE2 displayed defective binding activity (0.1–0.2% of apoE3's binding activity) (Table 1) (2, 22). In addition, the binding activity of the apoE2 22-kDa fragment (residues 1–191), which lacks the entire carboxyl-terminal domain, was 36-fold higher than that of intact apoE2 (3.6% of apoE3's activity, Table 1). Extending the carboxyl terminus from position 191 to 223 or 244 further reduced receptor binding to ~0.8% of apoE3's activity, as would be expected as the carboxyl terminus begins to exert its effect. Extension to position 272 reduced binding activity to the level of intact apoE2 (0.1–0.2% of apoE3's). These results suggest that the entire carboxyl terminus up to position 272 is required for complete modulation. As determined by negative-staining electron microscopy, apoE3 and apoE2 and their truncations formed DMPC discs of similar size (~15 nm in diameter). There were approximately three apoE molecules/disc for the intact proteins.

TABLE 1. Comparison of the receptor-binding activities of apoE2 and apoE-Leiden variants

Variant	Receptor-Binding Activity	
	ApoE2	ApoE-Leiden
	%	%
Intact	0.1 ± 0.2	25.0 ± 17.7
191 truncation	3.6 ± 1.4	104.7 ± 14.6
223 truncation	1.0 ± 0.5	21.5 ± 12.4
244 truncation	0.7 ± 0.1	16.4 ± 9.9
272 truncation	0.1 ± 0.2	—

Receptor-binding activity is expressed as a percentage of the binding activity of intact apoE3. Values are mean ± SD (n = 3).

Carboxyl-terminal truncation also affected the binding activities of apoE-Leiden variants (**Fig. 2**, Table 1). Consistent with the previous observations (7), intact apoE-Leiden, containing a small amount of apoE2 (9:1), displayed approximately 25% of apoE3's activity (Table 1). After removal of the carboxyl terminus (192–299), the 191 variant displayed an activity identical to that of apoE3 (7). However, truncation of the carboxyl terminus to position 223 or 244 did not significantly affect binding activity (i.e., both variants displayed a binding activity similar to that of intact apoE-Leiden).

Examination of the size of apoE-Leiden–DMPC discs by negative-staining electron microscopy revealed a previously unappreciated fact. Intact apoE-Leiden forms larger and more variably sized phospholipid discs than does apoE3 (23.6 ± 3.5 vs. 14.7 ± 1.8 nm) (**Fig. 3**). The phos-

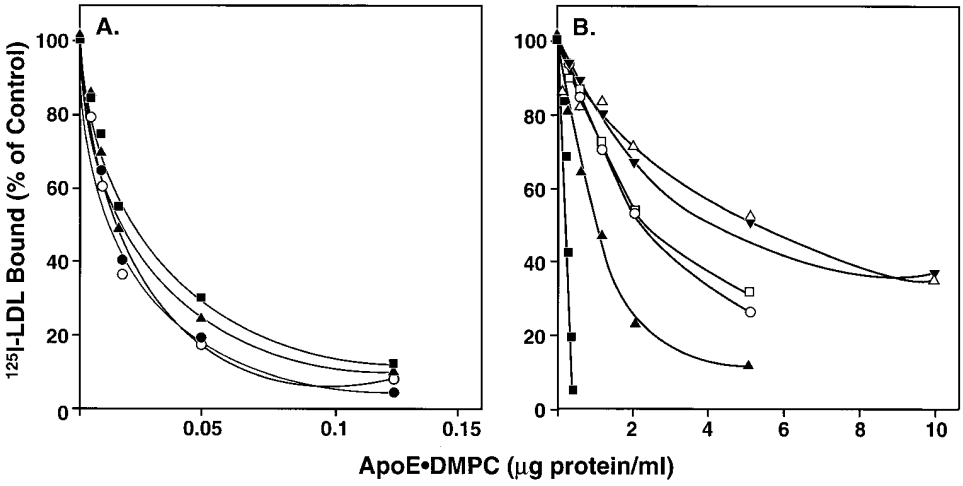

Fig. 1. Abilities of apoE–DMPC discs to compete with ^{125}I-labeled LDL for binding to LDL receptors on normal human fibroblasts. Before the binding assay, the various forms of apoE were complexed with DMPC at a ratio of 1:3.75 (w:w, protein:DMPC), and the complexes were isolated by ultracentrifugation in a linear KBr gradient from d 1.006 to d 1.21 g/ml (22). The fibroblasts were incubated at 4°C in a medium containing 2 μg/ml ^{125}I-labeled LDL and various concentrations of apoE·DMPC complexes. After a 2-h incubation, cells were washed extensively with cold phosphate-buffered saline containing 2 mg/ml bovine serum albumin and solubilized in 0.1 m NaOH. The ^{125}I content was determined in a Beckman 8000 gamma counter. (A) Intact apoE3 (■), apoE3-244 (○), apoE3-223 (●), and apoE3-191 (▲). (B) Intact apoE2 (▼), apoE2-272 (△), apoE2-244 (○), apoE2-223 (□), and apoE2-191 (▲). Data from one of the three experiments performed are shown. A summary of the three binding experiments is presented in Table 1.

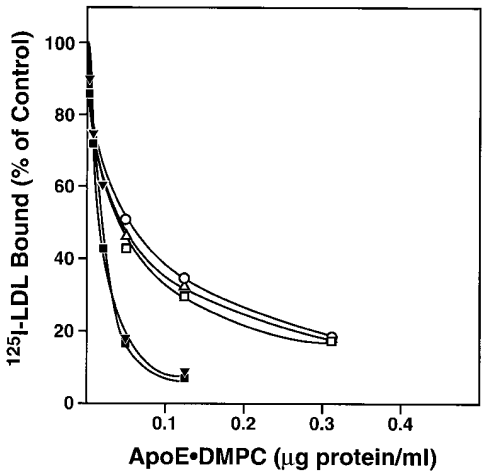

Fig. 2. Abilities of apoE-Leiden variants to compete with ^{125}I-labeled LDL to bind to the LDL receptor on normal human fibroblasts. The experiment was performed as described in the legend of Fig. 1. Data are shown from one of the three experiments performed. Data from all three binding experiments are summarized in Table 1. Intact apoE3 (■), intact apoE-Leiden (□), apoE-Leiden 244 (△), apoE-Leiden 223 (○), and apoE-Leiden 191 (▼).

pholipid:protein ratio of all the apoE-Leiden discs was similar (range 4.0–4.5) to that of the smaller apoE3 discs (4.4). The larger intact apoE-Leiden discs contained approximately six molecules/disc.

In addition, the sizes of the various apoE-Leiden discs were compared directly by gel filtration. Intact apoE3 formed one population of apoE-DMPC particles that eluted at 23 minutes (**Fig. 4A**). Intact apoE-Leiden from plasma (apoE-Leiden:apoE2 = 9:1) formed two populations (Fig. 4B). About 20% of the particles eluted at a position similar to that of intact apoE3 (23 min), while the majority of the discs eluted at an earlier position (18 min), indicating a larger size than the apoE3 discs. Analysis of each fraction by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) demonstrated that the first peak contained only apoE-Leiden and that the second peak contained a mixture of apoE2 and apoE-Leiden at a ratio of about 3:1, suggesting that the presence of a small amount of apoE2 resulted in the formation of a smaller disc. Consistent with this suggestion, recombinant intact apoE-Leiden formed only larger particles (Fig. 4C). The apoE-Leiden 191 variant (Fig. 4D) (and the 223 and 244 variants) primarily formed discs similar in size to the large discs formed by the intact apoE-Leiden (Fig. 4B and C). Therefore, these discs would be expected to contain 8–9 apoE-Leiden 191 molecules/disc. As a result, these particles would be expected to compete more effectively than normal-sized discs for binding to the LDL receptor.

The above results raise the possibility that previous estimates for both the receptor-binding activity of intact apoE-Leiden–DMPC particles (25% of normal binding activity) and apoE-Leiden 191–DMPC particles (normal binding activity) may not reflect their actual activities because both form larger DMPC particles with more molecules/disc than the control apoE3. To explore this possibility for intact apoE-Leiden, we used the VLDL-like microemulsion particles to compare directly the binding activities of apoE-Leiden and apoE3 on identically sized particles. ApoE3 and apoE-Leiden were incubated with microemulsion particles at concentrations determined to give similar amounts of particle-bound apoE. The protein:triglyceride ratios of the apoE3 and apoE-Leiden particles were 1:27.5 and 1:27, respectively. In the receptor-binding assay shown in **Fig. 5**, apoE-Leiden particles possessed approximately 12% of the activity of the apoE3 particles. The concentrations required for 50% replacement of ^{125}I-labeled LDL were 8.0 μg/ml for apoE-Leiden and 1.0 μg/ml for apoE3. The average binding activity of the apoE-Leiden particles from three experiments was 10.8% of the activity of the apoE3 particles. In a separate experiment, the binding activity of apoE2 on microemulsion particles was approximately 10% of the activity of apoE3, indicating that the binding activities of apoE2 and apoE-Leiden were similar in this system.

As apoE 191 fragments will not bind to VLDL-like microemulsion particles and as the apoE-Leiden 191 variant forms different size DMPC discs, we needed an alternative way to compare the binding activities of apoE3 and apoE-

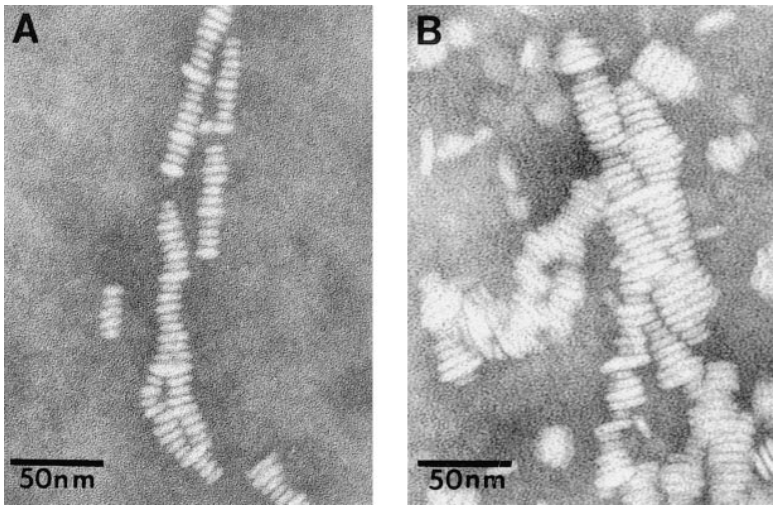

Fig. 3. Size comparison of apoE3–DMPC discs (A) and apoE-Leiden–DPMC discs (B) by negative-staining electron microscopy. The DMPC particles (0.06–0.08 mg/ml protein) were dialyzed against 125 mm ammonium acetate, 2.6 mm ammonium carbonate, and 0.26 mM EDTA (pH 7.4) and stained on the surface of carbon-filmed grids. Particle size was determined as described under Materials and Methods.

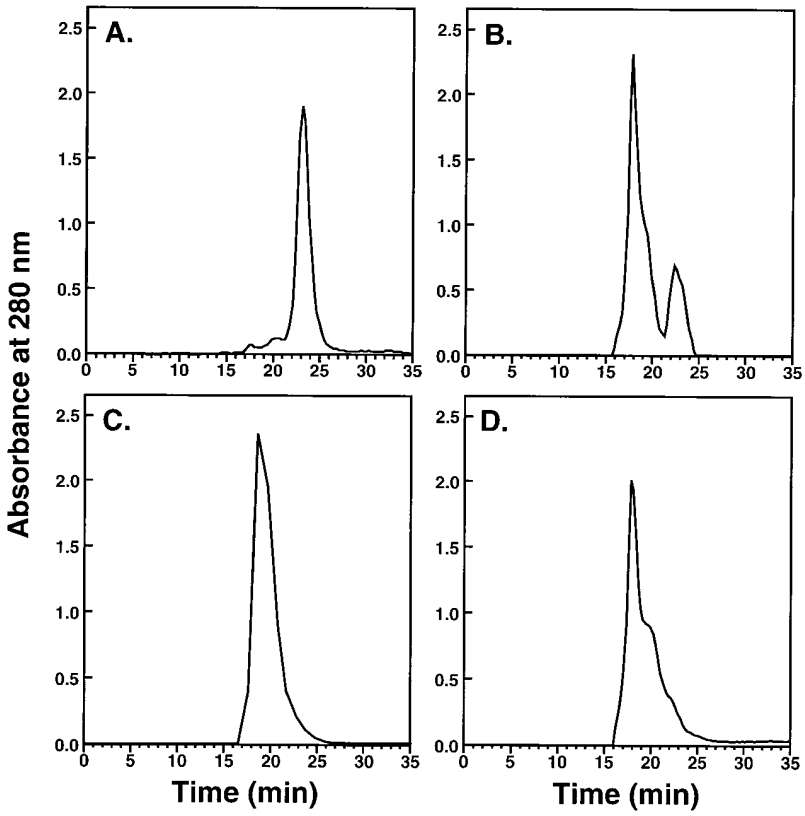

Fig. 4. Comparison of apoE–DMPC disc sizes by gel filtration on a Superdex 200 column. After equilibration of the column with 20 mm phosphate buffer (pH 7.4) containing 150 mm NaCl, 50 μl of the apoE–DMPC discs were applied to the column and eluted at 0.5 ml/min. The eluate was monitored at 280 nm. (A) ApoE3. (B) Intact apoE-Leiden isolated from plasma. (C) Recombinant intact apoE-Leiden. (D) ApoE-Leiden 191 variant.

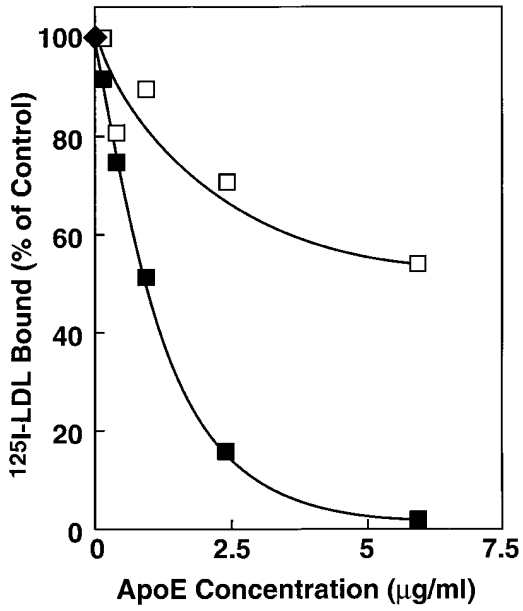

Fig. 5. Comparison of the receptor-binding activities of apoE3 and apoE-Leiden associated with VLDL-like microemulsion particles. ApoE3 (600 μg) or apoE-Leiden (400 μg) was incubated with the VLDL-like microemulsion particles at 37°C for 2 h. Particle-bound apoE was separated from free apoE on a Superose 6 column. The protein:triglyceride ratios of these particles are 1:27.5 for apoE3 and 1:27 for apoE-Leiden. The receptor-binding assay on normal human fibroblasts was performed as described in the legend of Fig. 1. ApoE3 (■) and apoE-Leiden (□).

Leiden 22-kDa fragments directly. We took advantage of a previous observation that lipid-free apoE bound to a plastic surface exhibits high-affinity binding to a soluble fragment of the LDL receptor (26). As shown in **Fig. 6**, the binding activity of the apoE-Leiden fragment in a solid-phase assay was 51.4 ± 9.4% of that of the apoE3 fragment. The apoE2 activity determined by this assay was 10.6 ± 8.4%, in agreement with previous results (26). This direct comparison demonstrated that the receptor-binding activ-

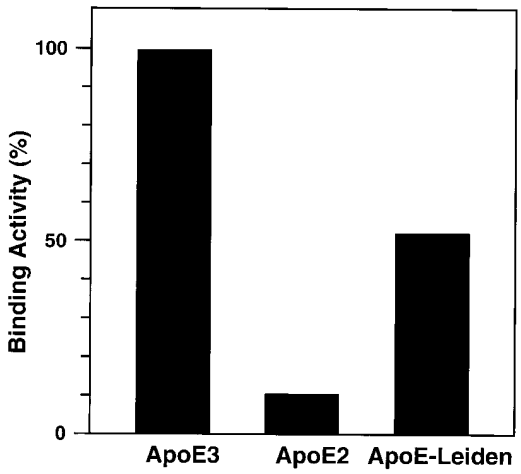

Fig. 6. Relative binding activity of apoE3, apoE2, and apoE-Leiden 22-kDa fragments to the LDL receptor in a solid-phase assay. Various apoE 22-kDa fragments (100 ng/well) were immobilized in microtiter wells and incubated with a soluble LDL receptor fragment. Binding was determined as described under Materials and Methods. Binding activity was normalized to 100% for apoE3.

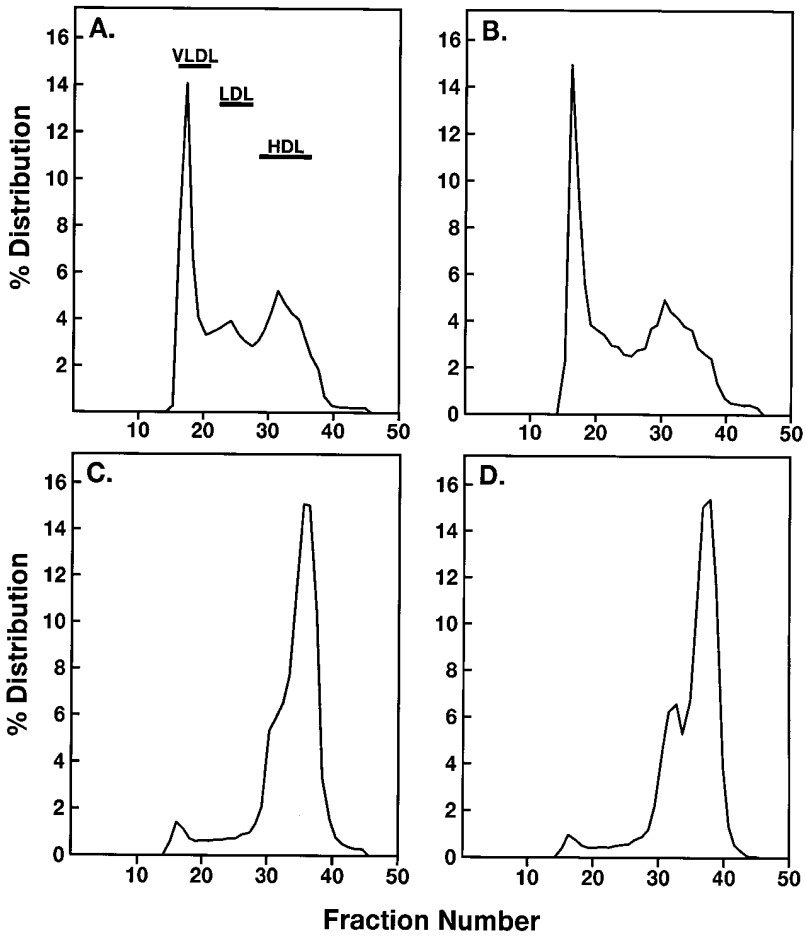

Fig. 7. Comparison of the plasma lipoprotein distributions of apoE4 and apoE-Leiden. After reduction with β-mercaptoethanol, 0.5–2 mg of iodinated apoE was incubated with 250 μl of normal human plasma at 37°C for 2 h. Sixty 0.5-ml fractions were collected, and the ^{125}I content in each fraction was determined by gamma counting. The elution positions for VLDL, LDL, and HDL are indicated by the bars. (A) Intact apoE4. (B) Intact apoE-Leiden. (C) ApoE4 191 variant. (D) ApoE-Leiden 191 variant.

ity of the apoE-Leiden 191 is defective compared with normal apoE3.

To determine whether intact apoE-Leiden and its 1–191 fragment have any other lipid-binding abnormalities, we compared the plasma distributions of apoE-Leiden and apoE4, both of which contain arginine at position 112. The distributions of apoE-Leiden and control apoE4 were essentially identical, both displaying a VLDL preference (**Fig. 7A** and **B**). In addition, the major portion of the apoE-Leiden 191 variant, like the apoE4 191 variant, eluted in the lipoprotein-free region of the elution profile beyond the high density lipoprotein (HDL) peak (Fig. 7C and D). These results indicate that lipoprotein-binding properties of the intact apoE-Leiden and its 1–191 fragment are not significantly different from those of intact apoE4 and its 1–191 fragment.

DISCUSSION

To examine the carboxyl-terminal modulation of the receptor-binding activity of apoE2 and apoE-Leiden, we applied a truncation approach used previously to map the major lipoprotein-binding regions of the carboxyl-terminal domain and the region involved in apoE4 domain interaction (17, 28). In contrast to previous studies in which discrete regions containing critical structural elements were identified (17, 28), this study shows that the entire carboxyl terminus of apoE2 up to position 272 appears to be required for complete modulation of receptor-binding activity. Sequential restoration of the carboxyl terminus from position 191 to 272 progressively reduced the binding activity to the level of intact apoE2 (less than 0.1% of apoE3's activity). The identical behaviors of the 272 variant and the intact protein suggested that the 272 variant contains all the structural elements required to modulate binding activity and that residues from 273 to 299 are not involved. This finding parallels our previous observation that residues 1–272 mimic the properties of the intact protein with respect to lipoprotein association and apoE4 domain interaction (17). Interestingly, sequence homology across species falls off beyond position 272, suggesting that this region of the protein is not involved in critical functions of apoE (4). Moreover, the carboxyl-terminal modulation of receptor-binding activity is restricted to apoE2, as similar truncations of apoE3 had no effect. The mechanism of this modulation is not clear. When apoE2 is combined with lipids, the carboxyl terminus likely influences the conformation of the receptor-binding region.

Previous studies with apoE-Leiden demonstrated that the intact protein displays ~25% of apoE3's binding activity and that removal of the carboxyl terminus increases the receptor-binding activity to a level identical to that of apoE3 (7). It was suggested that the seven amino acid re-

peat of apoE-Leiden on receptor-binding activity exerts its effect through the carboxyl terminus and that removing the carboxyl terminus released a structural constraint on the receptor-binding region (7), similar to the constraint suggested for apoE2 (14). However, the data from the present study indicate that the defective binding of apoE-Leiden is due to a direct effect of the seven amino acid repeat on the receptor-binding region rather than to an indirect effect mediated through the carboxyl terminus. Truncation to residues 244 and 223 had no significant effect on receptor-binding activity. Although further truncation to position 191 increased binding activity to normal levels (Table 1), as previously observed, we now believe that this increase in binding actually results from the very large DMPC discs formed by the 191 variant, which contain more molecules of apoE than the apoE3 discs (8–9 vs. 3). Increasing the number of apoE molecules capable of binding to LDL receptors on DMPC discs increases the receptor-binding activity as a result of multiple interactions with receptors (27). We established that the binding activity of the apoE-Leiden 191 fragment was defective compared to that of apoE3 in a direct comparison in a lipid-free solid-phase assay. These results indicate that the apoE-Leiden 191 variant has defective receptor-binding activity (51.4% of normal) resulting from a direct effect of the seven amino acid repeat.

The mechanism of the formation of large DMPC discs by apoE-Leiden is not clear. However, it seems to be a specific feature for DMPC discs of apoE-Leiden. No gross abnormalities in its lipoprotein-binding properties were observed here or in previous studies (29). In addition, the preference of intact apoE-Leiden for VLDL indicates that the seven amino acid repeat does not interfere with the interaction of arginine-61 and glutamic acid-255, which is responsible for apoE4 domain interaction and the preference of apoE4 for VLDL (30).

It was previously estimated that the receptor-binding activity of intact apoE-Leiden was approximately 25% of apoE3's activity. However, as we now know that the intact protein also forms larger DMPC discs with more apoE molecules / disc, the actual receptor-binding activity of intact apoE-Leiden is likely much lower. Using VLDL-like microemulsion particles, we were able to estimate its binding activity more accurately. When particles containing similar amounts of apoE3 and apoE-Leiden were compared, the binding activity of apoE-Leiden particles was 12% of that of the apoE3 particles, suggesting that this is the true binding activity of apoE-Leiden. Comparison of plasma accumulation of apoE-Leiden with another binding-defective variant, apoE3 (Arg 112, Cys 142) (20% of apoE3's binding activity) is consistent with this lower estimate of binding activity. The apoE3 (Arg 112, Cys 142) accumulates in the VLDL fraction in a subject heterozygous for apoE3 at a ratio of 3:1 (31). However, in the VLDL fraction in apoE-Leiden heterozygotes, the apoE-Leiden accumulated at a ratio of more than 9:1 compared to apoE3 (7). That apoE-Leiden accumulates to higher levels in plasma than apoE3 (Arg 112, Cys142) suggests that the apoE-Leiden has a lower affinity for the LDL receptor than apoE3 (Arg 112, Cys 142). This difference in accumulation of apoE-Leiden is consistent with our new finding that apoE-Leiden possesses only 12% of the binding activity of apoE3.

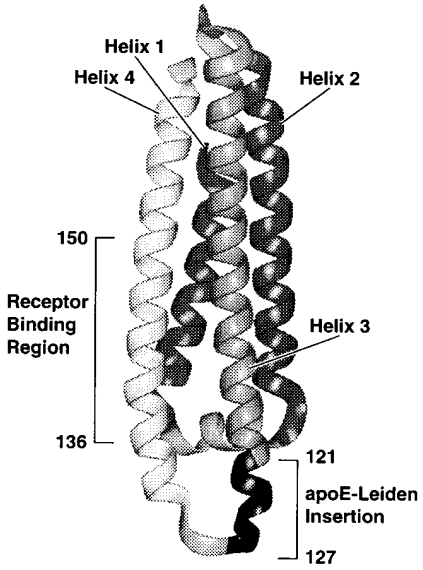

Fig. 8. Ribbon model of the four-helix bundle of the amino-terminal domain of apoE. Helix 1, residues 23–43; helix 2, residues 53–81; helix 3, residues 88–125; and helix 4, residues 130–165. Residues 121–127, which are repeated in apoE-Leiden, are highlighted in black.

One characteristic of the dominant variants associated with type III hyperlipoproteinemia is that their receptor-binding activities are permanently disrupted by mutations in the receptor-binding region (residues 136–150) (7). In contrast, apoE2 (Cys 158), with its mutation outside the receptor-binding region, is associated with recessive inheritance of this disorder. Its receptor-binding activity can be modulated in several ways, for example through the effects of charge modification (14), removal of the carboxyl terminus (14), or lipid composition (32). Based on the present studies, we conclude that receptor-binding activity of the apoE-Leiden is also "permanently" disrupted by the seven amino acid repeat. This conclusion is now consistent with the properties of other dominant variants.

How the seven amino acid repeat might affect the structure of the amino-terminal domain of apoE-Leiden and the receptor-binding region awaits detailed structural studies. As shown in **Fig. 8**, the insertion is located at the end of helix 3 (residues 88–125) and extends into the loop (residues 126–129) that connects helices 3 and 4 in the four-helix bundle of the amino-terminal domain (33). The insertion may result in extension of both helix 3 and the loop that could alter the structure of helix 4 (residues 130–165), which contains the receptor-binding region (residues 136–150).

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