Human apolipoprotein E7: lysine mutations in the carboxy-terminal domain are directly responsible for preferential binding to very low density lipoproteins

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Abstract **Apolipoprotein E7 (apoE7) (apoE3 E244K/E245K) is a naturally occurring mutant in humans that is associated with increased plasma lipid levels and accelerated atherosclerosis. It is reported to display defective binding to low density lipoprotein (LDL) receptors, high affinity binding for heparin, and like apoE4, preferential association with very low density lipoproteins (VLDL). There are two potential explanations for the preference of apoE7 for VLDL: lysine mutations, which occur in the major lipid-binding region (residues 244–272) of the carboxy-terminal domain of apoE7, could either directly determine the lipoprotein-binding preference or could interact with negatively charged residues in the amino-terminal domain, resulting in a domain interaction similar to that in apoE4 (interaction of Arg-61 with Glu-255), which is responsible for the apoE4 VLDL preference. To distinguish between these possibilities, we determined the binding preferences of recombinant apoE7 and two amino-terminal domain mutants, apoE7 (E49Q/E50Q) and apoE7 (D65N/E66Q), to VLDL-like emulsion particles. ApoE7 and both mutants displayed a higher preference for the emulsion particles than did apoE3, indicating that the carboxy-terminal lysine mutations in apoE7 are directly responsible for its preference for VLDL. Supporting this conclusion, the carboxy-terminal domain 12-kDa fragment of apoE7 (residues 192–299) displayed a higher preference for VLDL emulsions than did the wild-type fragment. In addition, lipid-free apoE7 had a higher affinity for heparin than did apoE. However, when apoE7 was complexed with dimyristoylphosphatidylcholine or VLDL emulsions, the affinity difference was eliminated. In contrast to previous studies, we found that apoE7 does not bind defectively to the LDL receptor, as determined in both cell culture and solid-phase assays. We conclude that the two additional lysine residues in the carboxy-terminal domain of apoE7 directly alter its lipid- and heparin-binding affinities. These characteristics of apoE7 could contribute to its association with increased plasma lipid levels and atherosclerosis.**—Dong, J., M. E. Balestra, Y. M. Newhouse, and K. H. Weisgraber. **Human apolipoprotein E7: lysine mutations in the carboxy-terminal domain are directly responsible for preferential binding to very low density lipoproteins.** ***J. Lipid Res.*** **2000.** 41: **1783–1789.**

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Apolipoprotein E (apoE) (299 residues, M_r = 34,200) plays a key role in lipoprotein metabolism (1, 2). It is a constituent of several classes of plasma lipoproteins (3) and serves as a high affinity ligand for the low density lipoprotein (LDL) receptor family, heparan sulfate proteoglycans (HSPG), and heparin. Through its interaction with LDL receptors and with the LDL receptor-related protein/HSPG pathway, apoE mediates the clearance of chylomicrons, very low density lipoprotein (VLDL), and their remnants from circulation (1, 4). There are two heparin-binding sites in apoE (5): a high affinity site in the amino-terminal domain (residues 140–150) and a low affinity site in the carboxy-terminal domain (residues 243–272), which is masked when apoE is associated with lipid. Basic amino acids in apoE (lysine and arginine) play a role in the binding of apoE to LDL receptors, heparin, and HSPG (5–7).

Three common isoforms of apoE exist in humans, apoE2, apoE3, and apoE4; they differ from each other by cysteine and arginine content at positions 112 and 158, which influences their respective biochemical and metabolic properties (8). In addition, a number of rare mutants are known. Both apoE3 and apoE4 bind with equally high affinity to LDL receptors, but apoE2 and several of the rare variants are defective in binding, which is an underlying cause of type III hyperlipoproteinemia, a lipid disorder characterized by the accumulation of remnant particles and accelerated atherosclerosis (9). The apoE isoforms are further distinguished by their binding preferences for various lipoprotein classes: apoE4 prefers large triglyceride-rich VLDL, whereas apoE3 and apoE2 prefer high density lipoproteins (HDL) (10–12). The preference of apoE4

Abbreviations: 2-ME, 2-mercaptoethanol; apoE, apolipoprotein E; BSA, bovine serum albumin; DMPC, dimyristoylphosphatidylcholine; EDTA, ethylenediaminetetraacetic acid; HDL, high density lipoproteins; HSPG, heparan sulfate proteoglycans; LDL, low density lipoprotein; PC, phosphatidylcholine; TO, triolein; Trx, thioredoxin; VLDL, very low density lipoprotein.

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for VLDL may contribute, by an unknown mechanism, to the increased plasma cholesterol and LDL levels and the increased risk for cardiovascular disease associated with this isoform (13).

The structural basis for the VLDL preference of apoE4 results from the interaction of the amino- and carboxy-terminal domains (13, 14). In apoE4, the arginine at position 112 changes the conformation of the Arg-61 side chain (amino-terminal domain), exposing it to solvent and placing it in a position to interact with Glu-255 (carboxy-terminal domain) (14). In apoE3, which has cysteine at position 112, Arg-61 is in a sheltered location where it cannot interact with Glu-255. The mechanism by which apoE4 domain interaction results in a preference for VLDL is not clear but it may be related to helical length (15). Domain interaction in apoE4 has been suggested to account for the unique biochemical and metabolic properties of this isoform (16).

ApoE7 is a naturally occurring mutant of apoE3 in humans, with two lysine residues replacing glutamic acid at positions 244 and 245 in the carboxyl terminus (17). This mutant is associated with hyperlipidemia and atherosclerosis and is reported to bind defectively to the LDL receptor, to bind with high affinity to heparin, and like apoE4, to associate preferentially with VLDL (18). Because apoE7 contains cysteine at position 112, it would not be expected to exhibit a domain interaction modulated by interaction of Arg-61 and Glu-255. Therefore, there are two potential explanations for the VLDL preference of apoE7. First, the mutations at positions 244 and 245 occur in the major lipid-binding region of apoE (residues 244–272) (14) and directly influence lipid-binding properties of apoE7. Second, these mutations may result in a domain interaction similar to that in apoE4 but involving different residues. Residues 244 and 245 are located 10 positions away from the Glu-255, and in addition, there are two pairs of negatively charged residues (Glu-49 and -50 and Asp-65 and Glu-66) located 10 positions away on either side of Arg-61 (**Fig. 1**). Thus, Lys-244 and -245 could interact with either Glu-49 and -50 or Asp-65 and Glu-66. In either case, neutralizing the negative charge at positions 49/50 or 65/66 in apoE7 would eliminate the lipoprotein preference of apoE7 for VLDL. To test this hypothesis, we produced recombinant apoE7, apoE7 (E49Q/E50Q), and apoE7 (D65N/E66Q) and determined their preferences for VLDL-like emulsion particles.

MATERIALS AND METHODS

Preparation of apoE7 DNA constructs

Point mutations were introduced into an apoE3 cDNA by site-directed mutagenesis and polymerase chain reaction with overlapping mutagenic oligonucleotide primers (Oligos Etc., Wilsonville, OR) (14). An apoE3 cDNA was subcloned into a modified thioredoxin (Trx) fusion protein expression vector (pET32a; Novagen, Madison, WI) at *Bam*HI and *Eco*RI restriction sites (Novagen) as described previously (19). All constructs were verified by DNA sequencing.

Expression and purification of recombinant apoE

The apoE-containing vectors were transformed into the T7 expression strain BL21 of *Escherichia coli* (Novagen), and transformants with high expression were grown in LB medium containing ampicillin (100 μg/ml) at 37°C to an optical density of 0.5–0.6 (550 nm). Expression was induced by adding isopropyl-1-thio-β-D-galactopyranoside (final concentration, 100 μg/ml) to the culture medium, and the cultures were grown for another 2 h.

Proteins were purified as described previously (19). Briefly, the bacteria pellet was sonicated, and the lysate was centrifuged to remove debris. The Trx fusion protein, which contains a histidine tag, was purified by His·bind metal chelation chromatography (Novagen). The fusion protein was then complexed with dimyristoylphosphatidylcholine (DMPC) at a ratio of 3.75:1 [DMPC–protein 3.75:1 (w/w)] and cleaved with thrombin (apoE–thrombin 100:1) (19). After inactivation of thrombin with 2-mercaptoethanol (2-ME), the mixture was lyophilized, delipidated with $CHCl_3$–methanol 2:1, solubilized in 6 M guanidine-HCl, 0.1 M Tris (pH 7.4), 1 mM ethylenediaminetetraacetic acid (EDTA), and 1% 2-ME, and purified by gel-filtration chromatography on a Sephacryl S-300 column in 4 M guanidine-HCl [0.1 M Tris (pH 7.4), 1 mM EDTA, 0.1% 2-ME]. Fractions containing apoE were pooled and dialyzed against 5 mM NH_4HCO_3 and lyophilized. Purified apoE was solubilized in 100 mM NH_4HCO_3 and stored at −20°C. The molecular weights of apoE7 and the variants were confirmed by mass spectrometry by

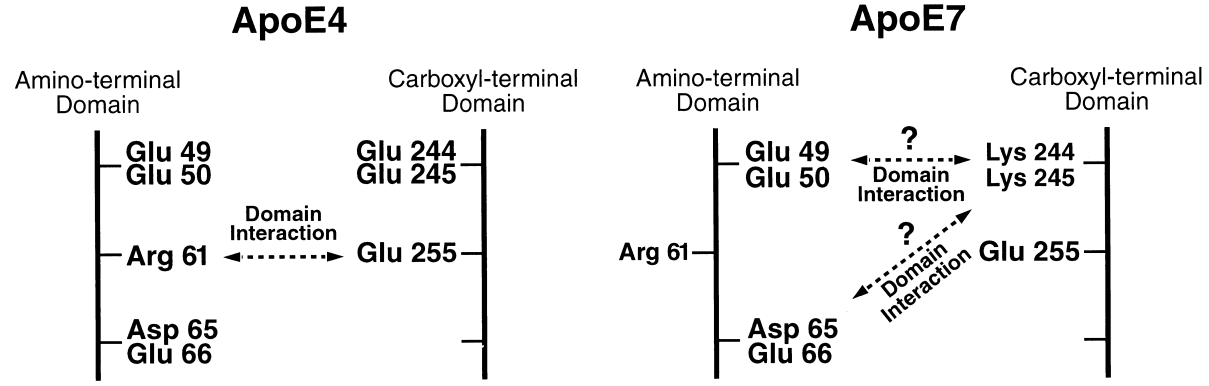

Fig. 1. Potential apoE4-like domain interaction in apoE7.

the University of California San Francisco Mass Spectrometry Facility and agreed within 2 mass units of the calculated molecular weights (data not shown). Isoelectric focusing was performed with 2% ampholines, pH 3.5–9.5 (Pharmacia, Uppsala, Sweden), as previously described (8). The 12-kDa fragments of apoE (residues 192–299) were prepared by thrombin digestion of full-length apoE as described (20).

Binding of apoE to VLDL-like emulsion particles

Binding of apoE to emulsion particles was assayed as described previously (13) with some modifications. Briefly, triolein (TO) (Sigma, St. Louis, MO) and egg yolk phosphatidylcholine (PC) (Sigma) [TO:PC 4:1 (w/w)] were mixed and dried under a stream of nitrogen. After resuspension in 10 mM Tris-KCl buffer (pH 8.0) containing 0.1 M KCl and 1 mM EDTA, the lipids were sonicated and centrifuged (21). After purification of the emulsion particles on a Superose 6 column, the lipid concentration was determined with enzymatic assay kits [from Wako (Tokyo, Japan) or Boehringer Mannheim Biochemicals (Indianapolis, IN)] according to the manufacturer instructions. ApoE (50 μg reduced with 0.1% 2-ME) was incubated with emulsion particles at a ratio of 1:10 [apoE–total lipid 1:10 (w/w)] for 2 h at 37°C. The emulsion-bound apoE was separated from unbound apoE with a TSK-4000 column (Pharmacia) eluted with 20 mM sodium phosphate containing 0.15 M NaCl (pH 7.0) at a flow rate of 0.5 ml/min. Fractions were collected and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting with a polyclonal antibody against human apoE as previously described (22).

LDL receptor-binding assays

Human LDL were isolated from plasma of normal fasting subjects by sequential ultracentrifugation (23) and radiolabeled by the iodine monochloride method (24). ApoE·DMPC disks were prepared as previously described and isolated by density-gradient ultracentrifugation (25). One week before the experiment, normal human fibroblasts were plated at 7.5×10^3 cells/22-mm 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 ^{125}I-labeled LDL (2.0 μg/ml) 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 (25).

LDL receptor binding was also measured with a solid-phase assay as described previously (26). ApoE3 or apoE7 (100 ng) in phosphate-buffered saline (20 mM sodium phosphate, 150 mM NaCl, pH 7.4) was incubated overnight at 4°C in 96-well microtiter plates (Dynatech Immulon; Dynex, Chantilly, VA). Nonspecific binding was blocked with 4% bovine serum albumin (BSA) in PBS for 1 h at room temperature. After each subsequent step, plates were washed with 1% BSA in PBS. Soluble LDL receptor fragment was diluted to approximately 10 ng/ml in PBS containing 3% BSA and 20 mM $CaCl_2$ and incubated with the apoE-coated wells for 2 h at room temperature. Bound receptor fragment was detected with anti-LDL receptor monoclonal antibody C7 (Amersham, Arlington Heights, IL) and horseradish peroxidase-labeled antimouse immunoglobulin G (Amersham), with color development with *o*-phenylenediamine dihydrochloride (Sigma) according to the manufacturer instructions. In parallel wells without added receptor, an anti-apoE antibody was used to determine the amount of apoE bound to normalize the results.

Determination of apoE·DMPC disk sizes

ApoE·DMPC complexes were applied to the surface of carbon fluid grids and negatively stained with 1% uranyl acetate. Electron micrographs were made at a magnification of ×200,000 and imported with a video camera into an Image 1/AT image-analysis system. Particle size was measured by automated sizing and counting programs available on system software (version 4.03a; Universal Imaging, West Chester, PA).

Determination of heparin-binding activity

Various forms of apoE or fragments (50 μg of protein) were applied to a 1-ml Hi-Trap heparin affinity column (Pharmacia) equilibrated with 20 mM Tris, pH 7.4. The bound proteins were eluted at a flow rate of 1 ml/min with a salt gradient (0–1 M NaCl). The elution profile was monitored by measuring the absorbance at 280 nm.

RESULTS

Recombinant apoE7 and apoE7 mutants were characterized by isoelectric focusing (**Fig. 2**). As expected, relative to apoE3, apoE7 was shifted four charge units in the basic direction, whereas apoE7 (E49Q/E50Q) and apoE7 (D65N/E66Q) were shifted six positive charge units. These results, along with the molecular weight determinations by mass spectrometry, confirmed the identity of the mutants.

Binding of apoE7 to VLDL-like emulsion particles

The relative binding of the various forms of apoE to VLDL-like emulsion particles is shown in **Fig. 3**. Compared with apoE3, apoE7 displayed preferential binding to the emulsion particles (82% vs. 61%), similar to that of apoE4 (84%). To determine whether domain interaction was responsible for the binding preference of apoE7 for the emulsion particles, apoE7 (E49Q/E50Q) and apoE7 (D65N/E66Q) were examined. ApoE7 (E49Q/E50Q) and apoE7 (D65N/E66Q) displayed binding preferences (both 84%) similar to those of apoE4 and apoE7. These data indicate that there is no domain interaction between Lys-244 and -245 and either Glu-49 and -50 or Asp-65 and Glu-66. Therefore, the lysine mutations appeared to be directly responsible for apoE7's preference for the VLDL emulsion particles.

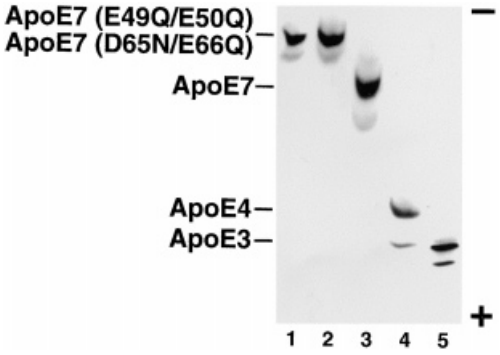

Fig. 2. Isoelectric focusing of apoE3, apoE4, apoE7, and apoE7 mutants. Proteins (10 μg) were focused on a polyacrylamide gel containing 8 M urea and 2% ampholines, pH 3.5–9.5, and stained with Coomassie Brilliant Blue R250: lane 1, apoE7 (E49Q/E50Q); lane 2, apoE7 (E65N/E66Q); lane 3, apoE7; lane 4, apoE4; lane 5, apoE3.

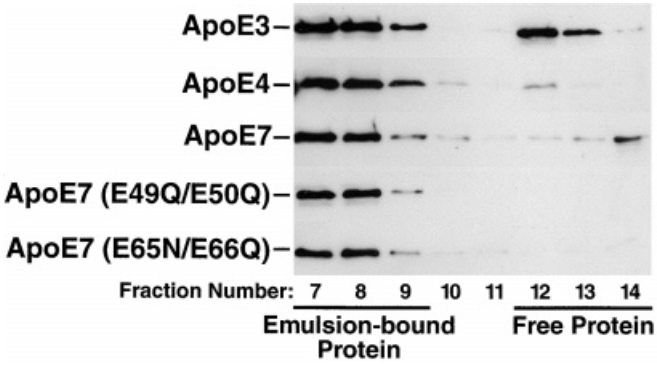

Fig. 3. Association of apoE3, apoE4, apoE7, and apoE7 mutants with VLDL-like emulsion particles. ApoE3, apoE4, apoE7, and apoE7 mutants (50 μg) were incubated with VLDL-like emulsions (1:10, protein/lipid, w/w) at 37°C for 2 h. Emulsion-bound apoE was separated from unbound apoE on a TSK 4000 column, eluted with 20 mM PBS at a flow rate of 0.5 ml/min. Emulsion-bound apoE eluted in fractions 7–9, while the free apoE eluted in fractions 12–14. Fractions were examined by sodium dodecyl polyacrylamide gel electrophoresis and immunobloting. Immunoblots were scanned on a Bio-Rad Molecular Imager and bands quantified with Bio-Rad Quantity One (version 4.1). Data presented are from one of two experiments, with two different batches of apoE mutant proteins. The results from both experiments were essentially identical.

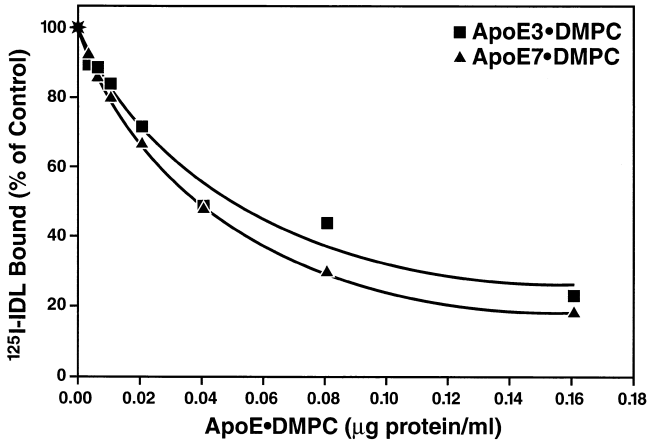

Fig. 5. Comparison of the abilities of apoE3 and apoE7·DMPC complexes to compete with ^{125}I-labeled LDL for binding to LDL receptors on normal human fibroblasts. ApoE3 and apoE7 were complexed with DMPC at a protein-to-DMPC ratio of 1:3.75 (w/w), and the complexes were isolated by ultracentrifugation in a linear KBr gradient from d = 1.006 to d = 1.21 g/ml. The fibroblasts were incubated at 4°C in a medium containing ^{125}I-labeled LDL at 2 μg/ml and various concentrations of apoE·DMPC complexes. After a 2-h incubation, the cells were extensively washed, and the amount of ^{125}I-labeled LDL bound to the cells was determined. Data presented are from one of three experiments.

To test this hypothesis, we assessed the ability of the carboxy-terminal 12-kDa fragments of apoE3 and apoE7 to bind emulsion particles. As shown in **Fig. 4**, the apoE7 fragment displayed a stronger preference for the emulsions than the apoE3 fragment (90% vs. 38%). These results indicate that the two lysine mutations in the carboxyl terminus are directly responsible for the VLDL preference of apoE7.

LDL receptor-binding activity of apoE7

The receptor-binding activity of apoE was determined first by measuring the ability of apoE·DMPC complexes to compete with ^{125}I-labeled LDL for binding to LDL receptors on cultured human fibroblasts. The concentration of apoE7·DMPC required for 50% displacement of the ^{125}I-labeled LDL in three experiments was 0.04 μg of protein per ml, while that for apoE3·DMPC was 0.05 μg of protein per ml (**Fig. 5**). The median effective dose of apoE7 in three experiments was 125 ± 18% of apoE3. Because this difference is within the normal variability of the assay, we regard the binding activities of apoE3 and apoE7 as similar, if not identical, but clearly not defective.

A previous study reported that apoE7 binds defectively to the LDL (23% apoE3's binding activity) (18), Therefore, we sought possible explanations. One important consideration in performing receptor-binding assays with

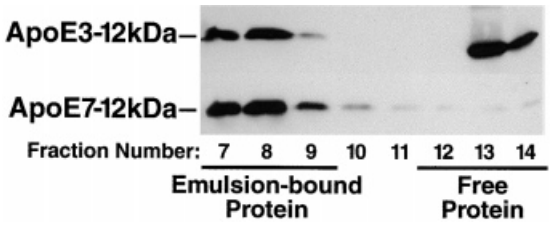

Fig. 4. Association of apoE3 and apoE7 carboxy-terminal domain (12 kDa) with VLDL-like emulsion particles. ApoE3 12-kDa and apoE7 12-kDa fragments were incubated with VLDL-like emulsions (1:10, protein/lipid, w/w) at 37°C for 2 h. Emulsion-bound protein was separated from unbound protein on a TSK-4000 column and protein quantitated as described in Fig. 3. Data presented are from one of two experiments; the results from both experiments were essentially identical.

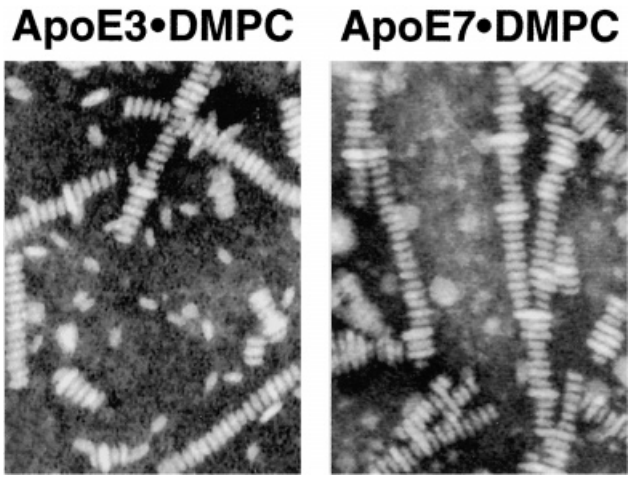

Fig. 6. Comparison of the size of apoE3·DMPC and apoE7·DMPC complexes by negative-staining electron microscopy. The apoE·DMPC particles isolated by ultracentrifugation were dialyzed against 125 mM ammonium acetate, 2.6 mM ammonium carbonate, and 0.26 mM EDTA (pH 7.4) and stained on the surface of carbon-filmed grids. Particle size was determined by Image 1 analysis.

DMPC complexes is that the disks be of similar sizes and contain a similar number of apoE molecules to allow for a direct comparison of binding activities. Disk diameters were not reported in the previous study. As shown in **Fig. 6**, the apoE3 and apoE7 disk diameters, as determined by negative-staining electron microscopy, were similar (14.3 ± 2.2 nm vs. 14.5 ± 2.5 nm). In addition, the phospholipid-to-protein ratios in both complexes were also similar (4.4:1 and 4.6:1, respectively). Because the disks were similar in the number of apoEs, we conclude that in our assay apoE7 has normal receptor-binding activity.

To confirm this finding with a second type of assay, a solid-phase receptor assay was performed with lipid-free protein. Previously, we showed that lipid-free apoE bound to the surface of microtiter wells displays high affinity binding to a soluble LDL receptor fragment containing the ligand-binding domain (26). Presumably, when bound to a plastic surface apoE assumes a conformation that mimicks its conformation on a lipid surface, which is required for high affinity binding to LDL receptors. This assay also has the advantage that considerations regarding variations in disk size or composition are eliminated. The average binding activity of apoE7 in 11 separate experiments was 110.6 ± 12.4% of the activity of apoE3. Thus, both the cell culture and solid-phase LDL receptor assays indicated that the receptor-binding activity of apoE7 is normal.

Heparin-binding activity of apoE7

Next, the relative heparin-binding activities of apoE3, apoE7, apoE3-12kDa, and apoE7-12kDa were determined in an assay that measures the relative contribution of electrostatic interactions of apoE binding to heparin. The proteins were applied to a heparin affinity column, bound protein was eluted with an NaCl gradient, and the relative binding affinities of various apoE variants to heparin were estimated. Significant differences in the elution positions of apoE3 and apoE7 were observed. ApoE7 was eluted from the column at a higher NaCl concentration than apoE3 (**Fig. 7A**), and the apoE7-12kDa fragment eluted at a higher NaCl concentration than the apoE3-12kDa frag-

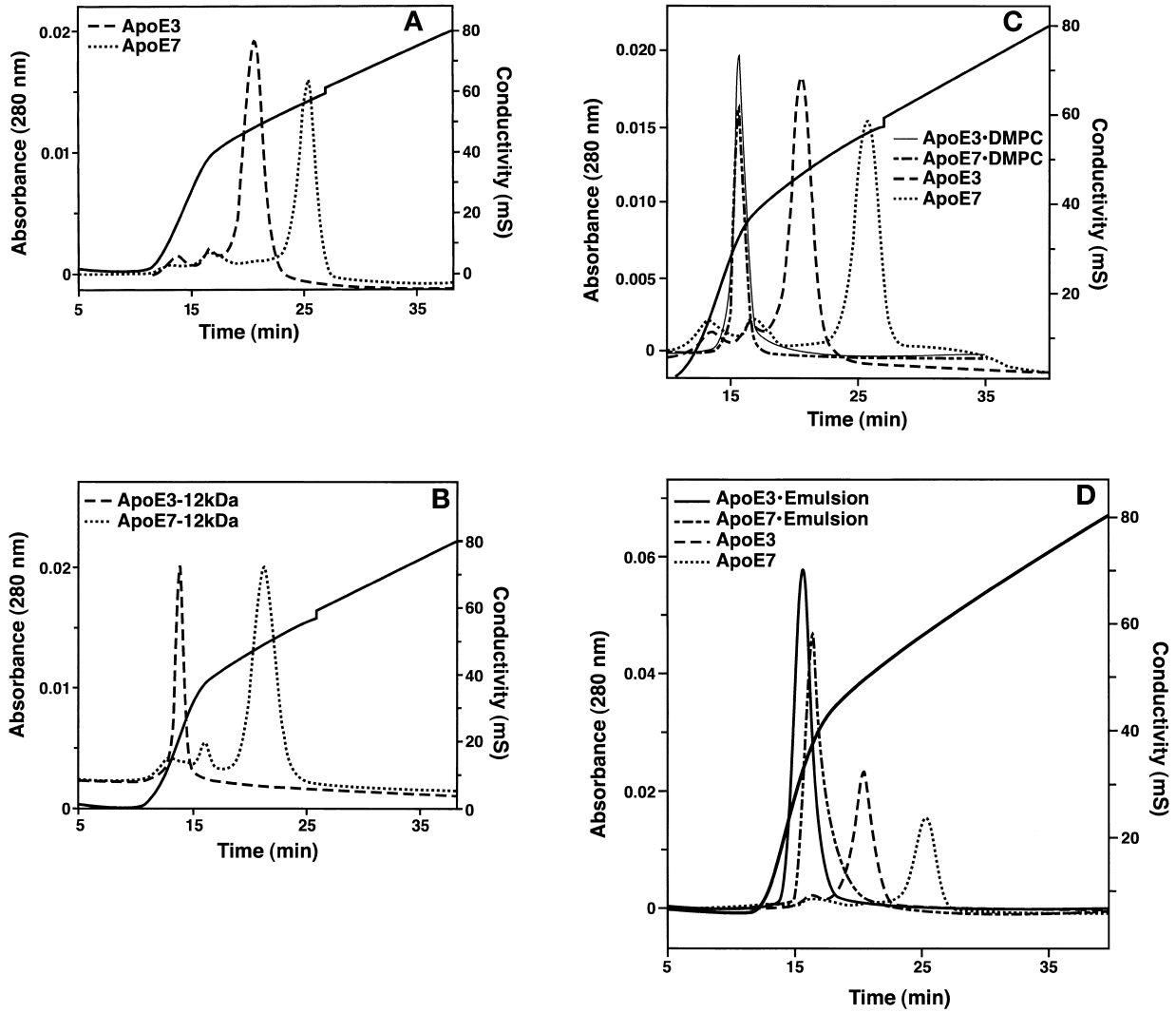

Fig. 7. Elution profiles of apoE bound to a heparin affinity column. Various forms of apoE were applied to heparin affinity column, equilibrated with 20 mM Tris, pH 7.4, and the bound apoE was eluted with a linear NaCl gradient (0–1.0 M). (A) ApoE3 versus apoE7; (B) apoE3-12kDa versus apoE7-12kDa; (C) apoE3·DMPC versus apoE7·DMPC; (D) apoE3-VLDL emulsion versus apoE7-VLDL emulsion.

ment (Fig. 7B). These results indicate that apoE7 has higher heparin-binding affinity than apoE3 and that the carboxy-terminal lysine mutations in apoE7, which occur within the second heparin-binding site, are responsible for the higher binding affinity.

To determine whether the second heparin-binding site of apoE7 is masked by lipids, as it is in apoE3 (5), we examined complexes of apoE3 and apoE7 with DMPC and VLDL-like emulsion particles. ApoE3·DMPC and apoE7·DMPC eluted at identical NaCl concentrations, which were lower than those at which their lipid-free forms eluted (Fig. 7C). Similar results were obtained with the apoE-VLDL emulsion particles (Fig. 7D). Combined, these results suggest that the second heparin-binding site in apoE7, like that in apoE3, is masked when complexed with DMPC or VLDL emulsions.

DISCUSSION

The results from our mutagenesis studies and binding assays using VLDL-like emulsion particles demonstrate that domain interaction is not responsible for the VLDL preference of apoE7. Rather, the preference is determined by the carboxy-terminal 12-kDa fragment (residues 192–299), suggesting that the double glutamic acid-to-lysine mutation in the major lipid-binding region affects the VLDL preference of apoE7 directly.

The carboxyl terminus of apoE contains two types of amphipathic helixes. Residues 203–266 form a class A helix, while residues 268–289 form a class G* helix (15). Class A amphipathic helices are the major lipid-binding structures in soluble apolipoproteins. Class A helices display unique clustering of positively charged amino acid residues at the polar-nonpolar interface and negatively charged residues at the center of the polar face (27). The position of basic residues at the interface has been suggested to stabilize amphipathic helical structures by a "snorkeling" mechanism (15), which is thought to allow the alkyl portion of the lysine and arginine side chains at the interface to penetrate deeper into the lipid hydrophobic layer, with the positively charged head group extending out toward the hydrophilic face. However, nuclear magnetic resonance studies with various apolipoproteins associated with sodium dodecyl sulfate suggest that snorkeling of basic residues may not be a general feature of apolipoproteins (28–30). Modeling of the class A amphipathic helix (residues 203–266) containing two lysines at positions 244 and 245 indicates that they are located at the center of the hydrophilic face, and thus involvement of a snorkeling-type mechanism appears unlikely. While it is not obvious exactly how the lysine mutations may favor binding to VLDL-like surfaces, we speculate that the mutations in some way stabilize the long putative class A helix (residues 203–266). It has been suggested that long amphipathic helices may be better accommodated on flatter surfaces (i.e., VLDL), and shorter helices may be better accommodated on more curved surfaces (i.e., HDL) (15).

The increased plasma lipid levels in subjects with apoE7 has been attributed to its defective receptor binding (18). However, our findings in this study show that apoE7 binds normally to the LDL receptor in both cell culture and solid-phase assays. In a previous report, apoE7 purified from subjects with an E7/3 phenotype had only 23% of normal binding activity (18). We used recombinant apoE7 containing an amino-terminal extension of two amino acids (glycine-serine). However, these additional residues do not affect receptor-binding activity; the properties of recombinant apoE are identical to those of apoE purified from plasma (19). Therefore, we do not believe that the additional two amino acids account for the difference in the two studies. The basis for the difference in LDL receptor-binding activity in the two studies remains unclear.

Because apoE7 binds normally to LDL receptors, its effects on lipid levels and atherosclerosis likely are related to its binding preference for VLDL. Therefore, the mechanism of these effects may resemble those responsible for the effects of apoE4. Potential mechanisms suggested for apoE4 include *i*) increased chylomicron uptake by the liver (31), resulting in either downregulation of LDL receptors and increased plasma concentrations of LDL (32) and/or stimulation of VLDL production (33); *ii*) conversion of VLDL to LDL; and *iii*) inefficient clearance of small VLDL and intermediate density lipoproteins as a result of LDL receptor downregulations.

We have confirmed the higher heparin-binding affinity of apoE7 compared with apoE3, reported previously (18). Studies using monoclonal antibodies and peptides have shown that apoE contains two heparin-binding sites, residues 142–147 and 243–272 (5). The first site corresponds to the LDL receptor-binding domain of apoE and is recognized by heparin in both the lipid-free and lipid-bound states. The second binding site is recognized by heparin only when apoE is lipid free (5). The increased heparin-binding activity of both apoE7 and its12-kDa fragment demonstrates that the mutations in the second heparin-binding region of apoE7 contribute to its higher heparin-binding affinity. The two positively charged lysines may interact directly with negatively charged groups on heparin and increase the binding affinity.

It has been suggested that the increased heparin or HSPG binding of apoE7 may contribute to the increased risk of atherosclerosis (18). The interaction of apoE-containing lipoproteins with proteoglycans in the arterial wall may be related to the deposition of lipids in atherosclerotic lesions (34, 35). However, our results indicate that, when apoE7 is complexed with either DMPC or VLDL-like emulsion particles, its second heparin-binding site is totally masked, as shown previously for apoE3 (5). This could result from burial of the second site in the lipid bilayer or from conformational change in the site induced by the interaction with lipid. However, it is not clear that the second heparin-binding site of apoE is masked on all classes of lipoproteins, in particular, atherogenic lipoproteins. Studies with human VLDL indicate that both binding sites are exposed on human VLDL (36). Thus, additional studies are required to address the interaction of circulating apoE7-containing lipoproteins with arterial wall glycosylaminoglycans.

In summary, we have demonstrated that the preference of apoE7 for VLDL is based on a direct effect of the two lysine mutations at positions 244 and 245 on the lipid-binding properties of the carboxy-terminal domain rather than an apoE4-like domain interaction. On the basis of our observation that the LDL receptor-binding activity of apoE7 is not defective, as previously reported, we propose that its impact in elevating plasma lipid levels and, in part, its effect on atherosclerosis likely involve mechanisms suggested for apoE4.

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