Characterization of apolipoprotein E7 (Glu_{244}→Lys, Glu_{245}→Lys), a mutant apolipoprotein E associated with hyperlipidemia and atherosclerosis

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Abstract **Previously, a mutant apolipoprotein (apo) E, apolipoprotein E7 (Glu_{244}→Lys, Glu_{245}→Lys), has been identified in association with hyperlipidemia and atherosclerosis. To investigate the effects of its structural changes on lipoprotein metabolisms and its correlation with atherosclerosis, we characterized this mutant apoE with respect to its receptor-binding, heparin-binding, and lipoprotein association. In a competitive binding assay, apoE7·dimyristoylphosphatidylcholine displayed a defective binding to the low density lipoprotein (LDL) receptor. The concentration of apoE7 required for 50% displacement of ^{125}I-labeled LDL was 0.223 μg/ml, while that for apoE3 was 0.048 μg/ml. ApoE7 possesses only 23% of normal binding activity. To investigate the lipoprotein preference of apoE7, we determined the relative amounts of apoE7 in plasma lipoprotein fractions obtained by ultracentrifugation or gel filtration. Like human apoE4, apoE7 was preferentially associated with the very low density lipoproteins (VLDL). For determination of heparin-binding activity, apoVLDL was applied to a heparin-Sepharose affinity column and the bound materials were eluted with a salt gradient. The apoE7 was eluted at a higher NaCl concentration (157 mm) than apoE3 (126 mm), indicating that this mutant has a higher affinity for heparin than does apoE3. While the reduced receptor-binding activity indicates delayed clearance of the triglyceride-rich lipoproteins, the preferential association of apoE7 with larger-size lipoproteins and the stronger interaction with heparin may compensate, to some extent, for the delayed clearance of triglyceride-rich lipoproteins. The strong interaction with proteoglycans in the arterial wall seems to be one of the possible explanations for the association of apoE7 with atherosclerosis.—**Yamamura, T., L-M. Dong, and A. Yamamoto. **Characterization of apolipoprotein E7 (Glu_{244}→Lys, Glu_{245}→Lys), a mutant apolipoprotein E associated with hyperlipidemia and atherosclerosis. *J. Lipid Res.* 1999.** 40: **253–259.**

Supplementary key words apolipoprotein mutant • heparin binding • lipoprotein • LDL receptor • receptor binding

Apolipoprotein (apo) E is a polymorphic protein (1, 2). Three common apoE isoforms in human are designated as apoE2 (Cys-112, Cys-158), apoE3 (Cys-112, Arg-158), and apoE4 (Arg-112, Arg-158) (1, 2). The polymorphism of apoE influences the plasma cholesterol and low density lipoprotein (LDL) concentrations. ApoE4 is known to be associated with higher cholesterol and LDL concentrations, and has been implicated as one of the risk factors for cardiovascular disease (3, 4). Although apoE2 is generally associated with lower cholesterol and LDL levels, under certain circumstances, homozygosity of apoE2 is an underlying cause of type III hyperlipoproteinemia, a genetic disorder characterized by elevated cholesterol and triglyceride levels and accelerated coronary artery disease (3, 4).

Apolipoprotein E has several important biological functions, including lipoprotein receptor binding, heparin binding, and lipoprotein association (for review, see ref. 5). The structural changes influence these functions. The wild-type apoE3 is known to preferentially associate with high density lipoprotein (HDL) when with cysteine at position 112, whereas apoE4 is known to preferentially associate with very low density lipoprotein (VLDL) when with arginine at position 112 (6, 7). The apoE4 preference for VLDL has been suggested to at least partially explain the elevated cholesterol and LDL levels in apoE4-carrying subjects (7). With respect to the lipoprotein receptor binding, apoE3 and apoE4 bind equally, whereas apoE2 with cysteine at position 158 is defective (less than 1% of apoE3 or apoE4 activity) (8). As a consequence of this defective binding, subjects homozygous for apoE2 allele have a delayed clearance of remnant lipoproteins (9). This, combined with genetic, environmental, or hormonal influences, may result in recessive expression of type III hyperlipoproteinemia (9). Several other identified apoE variants with defective receptor-binding ability have been shown to have associations with impaired catabolism of

Abbreviations: apo, apolipoprotein; DMPC, dimyristoylphosphatidylcholine; ELISA, enzyme-linked immunosorbent assay; HDL, high density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein.

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remnant particles which leads to the dominant expression of type III hyperlipoproteinemia (9). In addition to its interaction with lipoprotein receptors, apoE also mediates the cellular uptake of remnant lipoproteins through its interaction with proteoglycans on the cell surface (10, 11).

Structurally, apoE has two independently folded domains which also represent two major functional domains (12, 13). The amino-terminal domain (residues 1–191) contains the receptor-binding region (residues 136–150) (14–16) and the carboxyl-terminal domain (residues 244–272) contains the major lipid-binding region (12, 13, 17, and 18). Although the amino- and the carboxyl-terminal domains of apoE are independently folded, they can interact with each other and influence each other's functional properties (17, 19). For instance, in apoE4, substitution of cysteine with arginine at position 112 in the amino-terminus influences its lipid-binding carboxyl-terminal domain, thereby resulting in apoE4's preference for VLDL (in contrast to the apoE3 preference for HDL) (6, 7, and 17). In addition, removal of the carboxyl-terminal domain in apoE2 increases its receptor-binding activity by 10-fold, suggesting that the carboxyl-terminal domain modulates the receptor-binding amino-terminal domain (20).

We have previously identified apoE7 (apoE-Suita), an apoE mutant unique in the Japanese population (21, 22) which is characterized by the presence of four more positive charges than are found in the wild-type apoE3 on isoelectric focusing gel (21, 22). A much higher frequency (3%) of apoE7 allele in patients with myocardial infarction than in a control group (0.5%) in our lipid clinic suggests a strong association of this mutant apoE with the development of atherosclerotic vascular diseases (22). Analysis of the genomic DNA obtained from an apoE7-carrying subject has identified double mutations with lysine replacing naturally occurring glutamic acid at positions 244 and 245 in the carboxyl-terminus (23), thereby explaining the addition of the four positive charges to the molecule. In the present studies, we characterized the mutant apoE7 with respect to its receptor binding, heparin binding, and distribution among plasma lipoproteins. Our results demonstrated that compared to apoE3, apoE7 possesses a lower affinity for the LDL receptor and higher affinity for heparin, and associates preferentially with larger-size lipoproteins.

MATERIALS AND METHODS

Apolipoproteins

Sera were collected from hyperlipidemic subjects with an E2/2, E3/3, or E7/3 phenotype. The VLDL fractions were separated by ultracentrifugation and then delipidated (24). After solubilization in 0.1 M Tris-HCl (pH 7.4) containing 6 M guanidine, 0.01% EDTA and 1% 2-mercaptoethanol, the apoVLDL was applied to a Sephacryl S-300 (Pharmacia Biotech, Uppsala, Sweden) column (1.6 cm × 200 cm) and eluted with 0.1 M Tris-HCl, pH 7.4, containing 4 M guanidine, 0.01% EDTA, and 0.1% 2-mercaptoethanol. The apoE fraction was collected, extensively dialyzed against 5 mM NH_4HCO_3, and lyophilized.

For isolation of apoE isoproteins, preparative isoelectric focusing was used as described previously (25). Briefly, the fractions containing apoE from column chromatography were subjected to isoelectric focusing on a soluble polyacrylamide gel containing 8 M urea and 2% Ampholine, pH 5–7 (Pharmacia Biotech, Uppsala, Sweden). After isoelectric focusing, the visible protein band in the gel corresponding to asialo apoE2 or apoE3 was cut out. The gel slice was solubilized by adding the reducing agent DTT. The apoE isoproteins were separated from the acrylamide monomers by heparin-Sepharose (Pharmacia Biotech, Uppsala, Sweden) affinity chromatography (21). As only limited materials were available, gel permeation was avoided for the isolation of the asialo apoE7 isoprotein. Analytical isoelectric focusing was carried out as previously described (21).

Receptor-binding assay

ApoE·dimyristoylphosphatidylcholine (DMPC) (Sigma Chemical Co., St. Louis, MO) complexes were prepared as described by Rall et al. (26). Human ^{125}I-labeled LDL was prepared by the method of Fielding et al. (27). Normal human fibroblasts were cultured and competitive binding of apoE·DMPC against ^{125}I-labeled LDL to the LDL receptor on normal human fibroblasts was assayed at 4°C on ice as described by Innerarity, Pitas, and Mahley (28).

Distributions of apoE among plasma lipoproteins

Sera were obtained from the subjects with an E4/3 phenotype or an E7/3 phenotype. Lipoproteins were separated either by ultracentrifugation (VLDL, $d < 1.006$; LDL, $1.006 < d < 1.063$; HDL, $1.063 < d < 1.21$ g/ml) (24) or gel filtration. In the gel filtration, 1 ml of serum was applied to a Sepharose 4B column (90 cm × 1.6 cm) and eluted with 2 mM phosphate buffer (pH 7.4) containing 0.2 M NaCl, 1 mM EDTA, and 0.01% Na_3N at a flow rate of 8 ml/h. The cholesterol and triglyceride in fractions were measured enzymatically using commercially available kits (Kyowa Medix Co., Tokyo, Japan and Wako Pure Chemical Industries, Osaka, Japan, respectively). To determine the amount of apoE in the fractions, an enzyme-linked immunosorbent assay (ELISA) was used to measure the apoE using horseradish peroxidase-conjugated IgG against human apoE. Briefly, the antibody was purified from anti-apoE goat antisera (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan) by a human apoE affinity column (29). The antibody–enzyme conjugate was prepared by coupling 2 mg of horseradish peroxidase (EC 1.11.1.7, Boehringer-Mannheim GmbH, Mannheim, Germany) to 4 mg of purified antibodies and a sandwich-type ELISA was developed as previously described (29). The fractions corresponding to VLDL, LDL, and HDL were pooled according to the distributions of lipids and apoE. To determine the amount of apoE in the column fractions, an enzyme-linked immunosorbent assay (ELISA) was used to measure the apoE using a horseradish peroxidase-conjugated IgG against human apoE.

The lipoprotein fractions, obtained by either ultracentrifugation or gel filtration, were dialyzed and delipidated. The apolipoproteins were dissolved in 10 mM Tris-HCl, pH 8.6, containing 8 M urea and 10 mM DTT, and the relative amounts of apoE isoproteins in VLDL, LDL, and HDL fractions were estimated by isoelectric focusing followed by immunoblotting.

Heparin binding of apoE

The VLDL was obtained from the sera from subjects with E3/3 and E7/3 phenotypes by ultracentrifugation and then delipidated (24). A heparin affinity column (2.5 cm × 1.0 cm) packed with heparin-Toyopearl gel (TOSOH, Tokyo, Japan) and equipped with fast protein liquid chromatography apparatus (Pharmacia Biotech, Uppsala, Sweden) was equilibrated with 10 mM Tris-HCl, pH 8.6, containing 8 M urea and 10 mM DTT. The apoVLDL was loaded onto the column and the bound materials were eluted at a flow rate of 30 ml/h with 64 ml of a linear NaCl

gradient from 0 mm to 500 mm. The heparin-binding activities of mutant apoE were estimated by determining the NaCl concentration at which apoE was eluted.

RESULTS

LDL receptor binding of apoE7

Asialo apoE was purified from human apoVLDL by preparative isoelectric focusing and heparin affinity chromatography. **Figure 1** shows the analytical isoelectric focusing of isolated apoE isoproteins. Each of the isoproteins migrates as a single band on the gel. The receptor-binding activity was estimated by measuring the ability of apoE·DMPC complexes to compete with ^{125}I-labeled LDL for binding to the LDL receptor on human fibroblasts. Human asialo apoE3 and apoE2 were used as controls.

ApoE3 was normal and apoE2 was defective in binding to the LDL receptor (**Fig. 2**), duplicating the results of the previous studies by Weisgraber, Innerarity, and Mahley (8). Although apoE7 was able to compete with ^{125}I-labeled LDL for binding to the LDL receptor, apoE3 had a stronger affinity for the LDL receptor (Fig. 2). The concentration of apoE7 required for 50% displacement of the ^{125}I-labeled LDL was 0.223 μg/ml, while that for apoE3 was only 0.048 μg/ml. Plasma apoE7 displayed only 23% of apoE3 binding activity. We also investigated the binding activity of the recombinant apoE7 and found it to be similar to that of plasma apoE7, at 29% of apoE3 activity (data not shown).

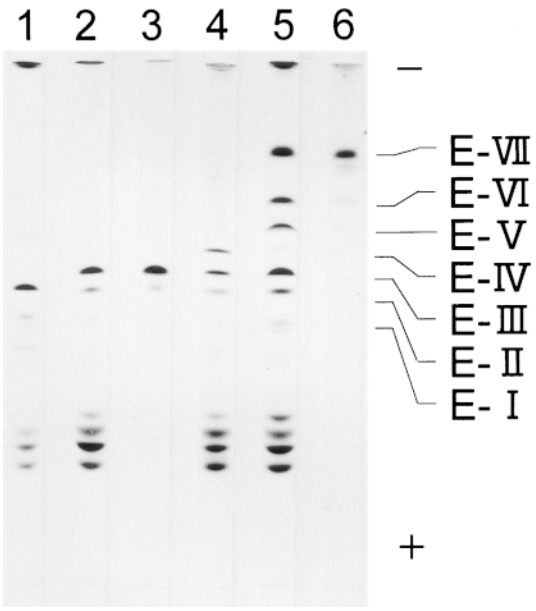

Fig. 1. Analytic isoelectric focusing on polyacrylamide gels (pH 3.5–8) of apoVLDL (lanes 1, 2, 4, and 5) and isolated apoE isoprotein (lanes 3 and 6). The apoE phenotypes shown are: lane 1, E2/2; lane 2, E3/3; lane 4, E4/3; lane 5, E7/3. The isolated apoE isoproteins shown are: lane 3, asialo apoE3; lane 6, asialo apoE7. The protein bands in the lower portions of the lane 1, 2, 4, and 5 are apoC isoforms. The cathode (basic) is at the top, and the anode (acidic) at the bottom.

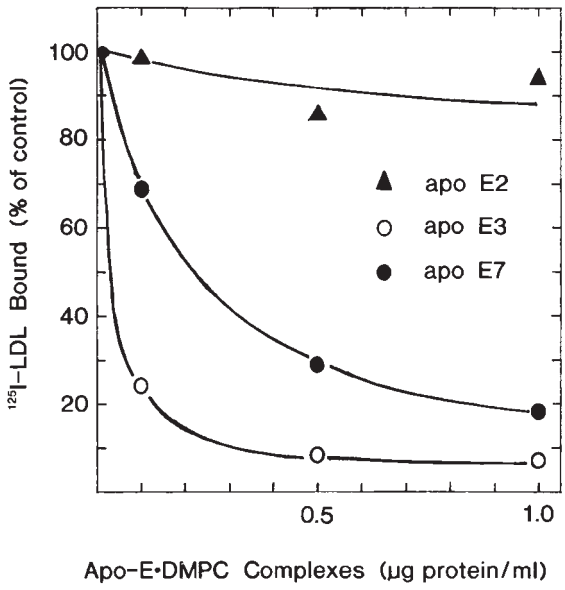

Fig. 2. Comparison of the binding activities of plasma apoE2 (▲), apoE3 (○), and apoE7 (●) to compete with ^{125}I-labeled LDL for binding to the LDL receptor on normal human fibroblasts. The cells were incubated in a medium containing ^{125}I-labeled LDL (2 μg/ml) and the indicated concentrations of apoE·DMPC complexes for 2 h at 4°C on ice. The cells were extensively washed and the ^{125}I-labeled LDL bound to the cells was determined. Each point indicates the average of duplicate dishes. ApoE2 (▲) was defective in its binding activity and apoE7 (●) displayed a poor binding activity compared to apoE3 (○).

Distribution of apoE7 among plasma lipoproteins

To determine the distribution of apoE7 among plasma lipoproteins, various lipoproteins were separated from serum by ultracentrifugation. The apoE distribution was analyzed by isoelectric focusing of apolipoproteins in each lipoprotein fraction followed by immunoblotting. For control, the distributions of apoE3 and apoE4 were also determined. In concordance with the previous reports (6, 7), apoE3 preferentially associated with HDL, and apoE4 preferentially associated with VLDL and intermediate density lipoprotein (IDL) (**Fig. 3**). As with apoE4, apoE7 was distributed slightly more in VLDL (lanes 2–4) and IDL + LDL (lanes 5–7) than in HDL (lanes 8–10) (Fig. 3). It is known that when ultracentrifugation is used to separate lipoproteins, apoE can be stripped off the larger lipoprotein particles at the bottom. To avoid this artificial effect, we next used gel filtration to separate the plasma lipoproteins (**Fig. 4**). As shown clearly in **Fig. 5**, apoE7 is more widely distributed in VLDL (lanes 2–4) and IDL+LDL (lanes 5–7) fractions than in HDL (lanes 8–10) fractions. These results demonstrated that despite apoE7's similarity to apoE3 in sharing cysteine at position 112, apoE7 is distributed more in larger-size lipoproteins and has a lipoprotein distribution pattern similar to that of apoE4.

Heparin-binding activities of apoE7

To estimate the heparin-binding activity, apoVLDL was applied to a heparin affinity column and the bound materials were eluted by an NaCl gradient. The elution profiles

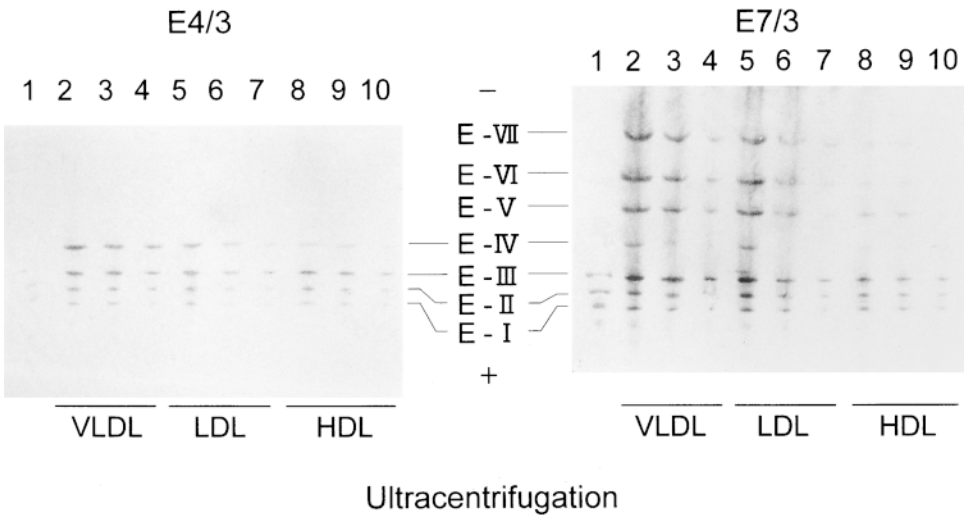

Fig. 3. Distribution of apoE4/3 and apoE7/3 among plasma lipoproteins separated by ultracentrifugation. Ultracentrifugal VLDL, LDL, and HDL were dialyzed and delipidated. The apolipoproteins were dissolved in 10 mm Tris-HCl buffer (pH 8.6) containing 8 m urea and 10 mm DTT, and subjected to isoelectric focusing (pH 3.5–8) followed by immunoblotting. Each lipoprotein fraction was electrophoresed in three lanes containing 60 ng, 40 ng, and 20 ng of apoE, respectively. Lane 1, control serum (E3/3 phenotype); lanes 2–4, VLDL; lanes 5–7, LDL; lanes 8–10, HDL.

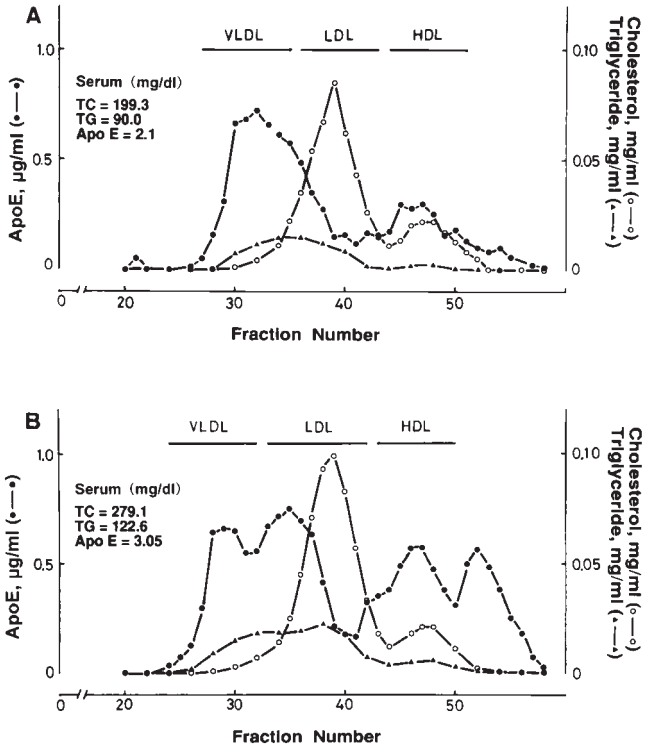

Fig. 4. Elution profiles of plasma lipoproteins from the subjects with an E4/3 phenotype (A) and an E7/3 phenotype (B) on a Sepharose 4B column. The concentrations of plasma cholesterol, triglyceride, and apoE were 199.3, 90.0, and 2.1 mg/dl, respectively, for the subject with an E4/3 phenotype, and 279.1, 122.6, and 3.05 mg/dl, respectively, for the subject with an E7/3 phenotype. The concentration of apoE in fractions was determined by ELISA, and the cholesterol and triglyceride in fractions were measured by enzymatic methods; (●) apoE; (○) cholesterol; (▲) triglyceride. Three fractions corresponding to VLDL, LDL, and HDL were separately collected.

of apoVLDL from a subject with an E3/3 phenotype and a subject with an E7/3 phenotype are shown in **Fig. 6**. There was only single peak (II) in the E3/3 profile, which was eluted at an NaCl concentration of 126 mm (Fig. 6A). However, the bound materials were eluted as two peaks (II′ and III′) in the E7/3 profile (Fig. 6B). The peak II′ in Fig. 6B was eluted at NaCl concentration (126 mm), as for peak II in Fig. 6A, whereas the peak III was eluted at a higher NaCl concentration (157 mm). The bound and unbound materials from heparin affinity chromatography (E7/3 phenotype) of Fig. 6B were fractionated and analyzed on isoelectric focusing (**Fig. 7**). The fraction II′, which is equivalent to the fraction II in Fig. 6A, contained mainly the E3 isoprotein. The fraction (d′) contained mainly the E7 isoprotein, while the fraction (c′) contained a mixture of apoE3 and apoE7 isoproteins. These results demonstrated that apoE7 has a higher affinity to heparin than apoE3.

DISCUSSION

In the present studies, we have characterized the apoE7 with respect to its receptor binding, heparin binding, and lipoprotein association. According to our results, this mutant displayed impaired binding (23% of apoE3 activity) to the LDL receptor, a higher binding affinity to heparin, and, like apoE4, a preferential association with VLDL.

The reduced receptor-binding activity of apoE7 was unexpected because the mutations of apoE7 occurred in the carboxyl-terminal domain rather than in the receptor-binding amino-terminal domain. Although the mechanism of the reduced receptor binding of apoE7 is not clear, the best possible explanation for this phenomenon is domain interaction, a new concept introduced by

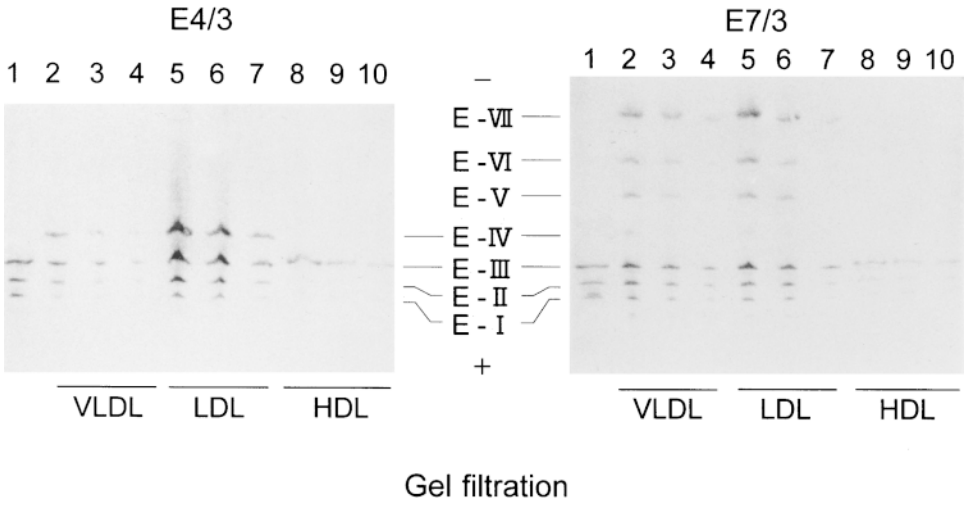

Fig. 5. Distribution of apo E4/3 and apo E7/3 among plasma lipoproteins separated by gel filtration. The analysis was performed as described in Fig. 3. and Fig. 4. Lane 1, control serum (E3/3 phenotype); lanes 2–4, VLDL; lanes 5–7, LDL; lanes 8–10, HDL.

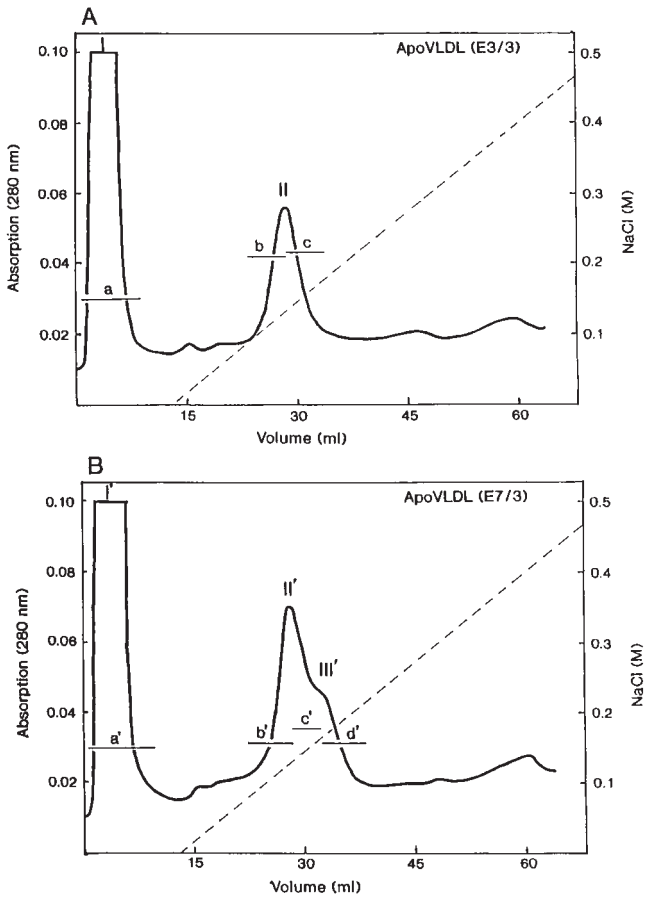

Fig. 6. Elution profiles of apoVLDL from a subject with an E3/3 phenotype (A) and an E7/3 phenotype (B) on a heparin-Toyopearl affinity column. Apo-VLDL in 10 mm Tris-HCl buffer, pH 8.6, containing 8 m urea and 10 mm DTT were applied to a heparin affinity column and eluted with 64 ml of a linear salt gradient from 0 mm to 500 mm NaCl at flow rate of 30 ml per h. The concentrations of NaCl at which peak II in (A), peak II′ and peak III′ in (B) were eluted were calculated. The fractions (a), (b), and (c) in (A), and the fractions (a′), (b′), (c′), and (d′) in (B) were collected and analyzed by isoelectric focusing.

Dong et al. (17) as a feature of apoE variants. The amino- and carboxyl-terminal domains have been suggested to interact with each other and influence each other's functions. For instance, position 112, a polymorphic site that distinguishes apoE3 and apoE4, lies in the amino-terminal domain, but the cysteine/arginine interchange at this position in apoE4 affects the property of the lipid-binding carboxyl-terminus and determines a preferential association with VLDL and IDL which contrasts with the preferential association with HDL in apoE3 (7, 17). In addition, removal of the entire carboxyl-terminus (191–299) of apoE2 (Cys–158) results in an activation of its receptor-binding activity by 10- to 20-fold, suggesting that the carboxyl-terminus modulates the receptor-binding amino-terminus (20). The fact that apoE7 with mutations in the carboxyl-terminus has a lower receptor-binding activity suggested that the carboxyl-terminal domain of apoE7 is likely to interact with the amino-terminus modulating its receptor binding. These findings provide another example to "apoE domain interaction." The modulation of receptor binding by the carboxyl-terminus in apoE7 is probably different from that in apoE2. In apoE2, the carboxyl-terminal modulation of the receptor binding requires a cysteine at position 158 (20), whereas that in apoE7, like apoE3, has arginine at this position. In addition to the domain interaction, it is also possible that the mutations in the major lipid-binding region (residues 244–272) (17, 18) alter apoE7's lipid-binding property (as will be discussed below), which in turn affects its receptor binding.

As discussed above, apoE isoproteins have isoform-specific preferences for lipoproteins. Among the three common isoforms, apoE3 and apoE2 prefer HDL and apoE4 prefers VLDL and IDL. Extensive study of the mechanism for apoE4 preference for VLDL has indicated that the domain interaction is likely to be responsible. In apoE4, it has been shown that the interchange of cysteine/arginine at position 112 results in a new salt bridge formation

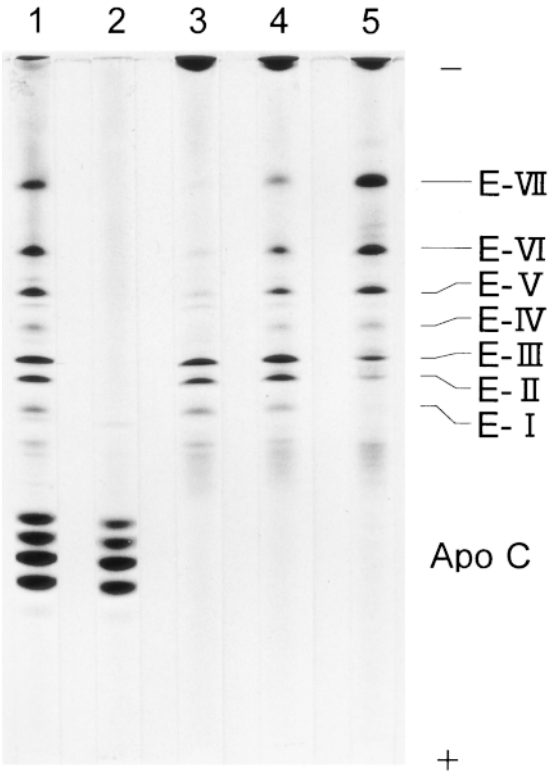

Fig. 7. Isoelectric focusing of apoVLDL and the fractions from heparin affinity chromatography (E7/3 phenotype). The proteins in the fractions a′, b′, c′, and d′ of Fig. 6B were focused on a polyacrylamide gel containing 8 m urea and 2% Ampholine, pH 3.5–8. Lane 1, apoVLDL with an E7/3 phenotype; lane 2, fraction a′ (heparin-Sepharose-unbound apoC); lane 3, fraction b′ (mainly apoE3); lane 4, fraction c′ (a mixture of apoE3 and apoE7); and lane 5, fraction d′ (mainly apoE7).

between glutamic acid 109 and arginine 112, and that the arginine 61 swings out to an ideal position for interacting with the lipid-binding carboxyl-terminal domain (17). A series of mutagenesis studies have suggested that arginine-61 in the amino-terminus and glutamic acid-255 in the carboxyl-terminus may interact with each other to direct apoE4 binding to VLDL (17, 30). Mutations of one of these two residues have been shown to alter the apoE4 preference from VLDL to HDL (17, 30). Although apoE7 has cysteine at position 112, the present studies have shown that, like apoE4, it is distributed preferentially to the larger-sized VLDL and IDL. There are two possible explanations for the apoE7 VLDL preference. First, the mutation at positions 244 and 245 occurred in the major lipid-binding domain (residues 244–272) (17, 18), which may change apoE7's lipid-binding property directly and alter its lipoprotein preference. Second, the mutations in the carboxyl-terminus may result in a domain interaction similar to that occurring in apoE4. It is known that apoE7, like apoE3, has cysteine at position 112, and thus the arginine-61 may not be available for the interaction with the glutamic acid-255 in the carboxyl-terminus. However, substitutions of the glutamic acids 244 and 245 for lysine residues in the carboxyl-terminus of apoE7 might result in an interaction of these positively charged residues with negatively charged residues such as the glutamic acids 50 and 51. If this does indeed occur, such an interaction could be expected to confer a conformation similar to that in apoE4 which would make apoE7 prefer the larger-size lipoproteins. Further studies are required to confirm these speculations.

The binding activities of apoE7 to heparin were also determined. ApoE7 displayed a stronger affinity for heparin than that displayed by apoE3. It has been suggested that apoE has two heparin-binding sites (31, 32). The major heparin-binding site coincides with the receptor-binding domain (residues 136–150) and the other one is located in the carboxyl-terminus in the vicinity of the residues 191–218 (31, 32). A recent study with an apoE mutant (Glu_{212}→Lys) has revealed that this substitution resulted in a higher binding affinity to both heparin and heparan sulfate on the cultured cell surface (about 20% higher than apoE3) (33). These results suggest that the region around residue 212 harbors the second heparin-binding site (33). The fact that apoE7 also binds to heparin with a higher affinity suggests that the positions 244 and 245 are also involved in the second heparin-binding site. Taken together, the second apoE heparin-binding site most likely spans the region from residue 210 to residue 245. It is not clear whether the second heparin-binding site of apoE plays a role in the physiological conditions. The results from studies using apoE·DMPC complexes or canine HDLc, in which apoE is the only protein constituent, have suggested that the second apoE heparin-binding site is masked when apoE is associated with lipid (31). However, the studies using human VLDL have indicated that both binding sites are expressed on human VLDL particles (33). It was speculated that the differences between these studies may be due either to the presence of not only apoE, but also apoB and apoCs in human VLDL, or to a conformational change induced by the mutation at position 212.

Several unrelated studies have shown a strong association of apoE7 with hyperlipoproteinemia and atherosclerosis (21, 22, 34, 35). The defective binding of apoE7 to the LDL receptor may delay the clearance of apoE7-containing lipoproteins and result in an accumulation of these lipoproteins in blood. However, its preferential association with larger triglyceride-rich lipoproteins and its stronger interaction with heparin may compensate, to some extent, for the delayed clearance of these lipoprotein particles. Therefore, persons carrying apoE7 are unlikely to develop severe hyperlipoproteinemia. This is consistent with previously published clinical data demonstrating that patients with apoE7 isoform had only mild hyperlipoproteinemia (22). This does not seem to be sufficient to explain the strong association of apoE7 allele with the development of atherosclerotic diseases.

The interaction of apoE with proteoglycans on the arterial wall has been suggested to be related to the deposition of cholesterol associated with atherosclerosis (36, 37). If the strong interaction of apoE7 with proteoglycans takes place on the artery wall, it may be one of the possible explanations for the association the apoE7 allele with atherosclerotic diseases. Further in vivo studies will be needed to investigate the mechanisms.

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