Apolipoprotein E distribution among human plasma lipoproteins: role of the cysteine-arginine interchange at residue 112

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Abstract Human apolipoprotein (apo) E occurs as three common isoforms (apoE4, E3, and E2), all of which influence plasma cholesterol levels. Although both apoE4 and E3 bind with equal effectiveness to the low density lipoprotein receptor, they associate preferentially with different classes of plasma lipoproteins: apoE4 with very low density lipoproteins, apoE3 with high density lipoproteins. The primary structure of apoE3 differs from that of apoE4 at only a single site; apoE3 has its sole cysteine residue at position 112, while apoE4 contains arginine at position 112 and completely lacks cysteine. The present study investigated how this structural difference between apoE4 and E3 determines their distribution among plasma lipoproteins, and analyzed the role of the disulfide-linked heterodimer apoE-A-II (which apoE4 cannot form) in determining the distribution. Human plasma was incubated with ^{125}I-labeled apoE, and lipoproteins were separated by agarose chromatography. Both apoE3 that had been reduced and alkylated with iodoacetamide and apoE3-A-II distributed with high density lipoproteins, indicating that a combination of an inherent property of the monomeric apoE3 structure and apoE-A-II formation account for distribution of apoE3 to the high density lipoproteins. Cysteamine modification of apoE3 resulted in an apoE4-like distribution, demonstrating that a positive charge at position 112 determined the apoE4 distribution and that the effect was not exclusively due to the presence of arginine at this position. The distribution of the 22-kDa thrombolytic fragment (residues 1–191) and the 12-kDa thrombolytic fragment (residues 192–299) of apoE indicated that while the carboxyl-terminal structural domain is the major lipid-binding region of apoE, the cysteine-arginine interchange at position 112 in the amino-terminal domain determines the lipoprotein class to which the carboxyl-terminal domain will bind. This implies a communication or interaction between the two apoE structural domains on a lipoprotein particle. —**Weisgraber, K. H.** Apolipoprotein E distribution among human plasma lipoproteins: role of the cysteine-arginine interchange at residue 112. *J. Lipid Res.* 1990. **31:** 1503–1511.

Supplementary key words domain interaction • structure-function • chemical modification • apoE-A-II

Apolipoprotein (apo) E is a constituent of several classes of plasma lipoproteins, and it plays a prominent role in cholesterol and triglyceride metabolism because of its ability to bind to the low density lipoprotein (LDL) receptor (1–3). Three common isoforms of apoE, designated E4, E3, and E2, occur in every population that has been studied, and the allelic frequencies are remarkably similar in these populations (4). This polymorphism results in six common phenotypes: three homozygous (E4/4, E3/3, and E2/2) and three heterozygous (E4/3, E4/2, and E3/2). Cysteine-arginine interchanges at residues 112 and 158 account for the structural differences among the three common isoforms: apoE4 has arginine at 112 and 158, and no cysteine; apoE3 has cysteine at 112 and arginine at 158; and apoE2 has cysteine at both sites (5, 6). The two cysteine-containing isoforms, apoE3 and E2, also exist in the plasma as apoE-A-II, a disulfide-linked heterodimer (7).

Plasma cholesterol and LDL concentrations are influenced by apoE polymorphism. It has been estimated that 60% of the variation in plasma cholesterol levels is genetically determined and that apoE polymorphism accounts for approximately 14% of this genetic variation (4). It has been demonstrated in several populations that the ϵ4 allele is associated with the highest cholesterol and LDL levels, the ϵ2 allele with the lowest, and the ϵ3 allele with the intermediate levels (8–12). Emerging data indicate that subjects with the ϵ4 allele are at the highest risk for developing cardiovascular disease (4).

Although the mechanism linking the ϵ4 allele with elevated plasma cholesterol levels is unknown, apoE4 has several special characteristics. Gregg et al. (13) have demonstrated in E4/4 and E3/3 homozygotes that apoE4 is metabolized faster than apoE3 in vivo and that apoE4 and E3 distribute differently among ultracentrifugal lipoprotein fractions. Relative to apoE3, more apoE4 is found

Abbreviations: apo, apolipoprotein; LDL, low density lipoproteins; VLDL, very low density lipoproteins; HDL, high density lipoproteins; IDL, intermediate density lipoproteins.

in very low density lipoproteins (VLDL) than in high density lipoproteins (HDL). This differential distribution has also been observed among plasma lipoproteins separated by agarose column chromatography in a subject with the E4/3 phenotype (14, 15). Chylomicron remnants are cleared faster in subjects with apoE4 than in those with only apoE3 (16).

The apoE4 distribution among plasma lipoproteins is thought to be a key component in understanding the mechanism by which apoE affects plasma cholesterol and LDL concentrations. The difference in plasma distribution between apoE4 and E3 has been suggested to result from either the fact that apoE4 has a positively charged arginine at 112 or that, unlike apoE3, apoE4 cannot form the heterodimer apoE-A-II (13, 14, 16). As a result of heterodimer formation, it was speculated, apoE3 would distribute preferentially to the HDL fraction (14).

In the present study, a direct biochemical test of these previously hypothesized mechanisms for the distribution properties of apoE4 and E3 in plasma was performed. The study was designed to distinguish experimentally between the role of charge at residue 112 and the formation of apoE3-A-II in determining the lipoprotein preferences of apoE4 and E3. Various forms of ^{125}I-labeled apoE were incubated with plasma, and agarose column chromatography was used to determine the distribution of labeled apoE among lipoprotein classes. The results demonstrate that although apoE-A-II distributes primarily in the HDL fraction, monomeric apoE3 also possesses this property, and that a positive charge at residue 112 determines the distribution of these isoforms. In addition, there appears to be interaction between the amino- and carboxyl-terminal domains of the protein, in that the charge on the amino acid at residue 112 influences which lipid surface the carboxyl-terminal domain will bind to.

MATERIALS AND METHODS

Blood was obtained from subjects fasted overnight and was collected in tubes containing EDTA (1 mg/ml of blood); plasma was prepared by centrifugation. The subjects' plasma concentrations of cholesterol and triglyceride were in the normal ranges for age- and sex-matched controls (17), as determined by using commercial enzyme kits (Abbott Laboratories, North Chicago, IL).

The apoE phenotype was determined by isoelectric focusing of delipidated plasma followed by immunoblotting, as described by Menzel and Utermann (18). Apolipoprotein E was isolated from d < 1.02 g/ml lipoproteins using Sephacryl S-300 chromatography as previously described (19). Thrombolytic fragments of apoE were prepared as described (20). Thrombin (specific activity, 2800 units/ml) was a gift from Dr. J. Fenton II of the New York Department of Health (Albany, NY). The intact protein and fragments were iodinated (specific activity of 250–2000 dpm/ng of protein) with ^{125}I-labeled Bolton-Hunter reagent (Amersham/Searle, Arlington Heights, IL) as described (21). Reduction and alkylation of cysteinyl residues with iodoacetamide or iodoacetic acid was performed by solubilizing the lyophilized protein (2–6 mg in 1–2 ml) in 6 M guanidine containing 2 mM EDTA, 20 mM dithiothreitol, and 0.1 M sodium phosphate, pH 7.8 (2 mg of protein/ml). After 4 h at room temperature, the mixture was chilled to 0°C, solid iodoacetamide (148 mg/ml) or iodoacetate (166 mg/ml) was added to it, and the mixture was allowed to stand for 30 min. Excess reagents were removed by dialysis against 0.1 M NH_4HCO_3. Cysteine alkylation was essentially complete, as determined by amino acid analysis (6). Cysteamine modification was performed by solubilizing apoE3 (3 mg) in 3 ml of 6 M urea containing 20 mM dithiothreitol, 10 mM Tris-HCl, pH 8.0, reducing at room temperature for 4 h, adding a 10-fold molar excess of cysteamine, and allowing the mixture to stand overnight at room temperature. Excess reagents were removed by dialysis against 0.1 M NH_4HCO_3.

Agarose chromatography was performed at 4°C on Bio-Gel A-5m (Bio-Rad, Richmond, CA) in Amicon glass columns (2.2 × 80 cm) (Amicon Corp., Lexington, MA), using a buffer of 0.15 M NaCl containing 10 mM sodium phosphate, pH 7.4. Iodinated apoE or apoE fragments (0.5–5 μg) were incubated with plasma (5 ml) for 2 h at 37°C before chromatography was begun. Columns were eluted at approximately 15 ml/h, and 3.6-ml fractions were collected. Cholesterol distributions were determined as described above, and ^{125}I activity was determined in a Beckman model 9000 gamma counter (Beckman Instruments, Fullerton, CA). Concentrations of apoE in column fractions were determined by an adaptation of the solid-phase radioimmunoassay procedure of Fainaru, Havel, and Imaizumi (22). With this modification, intra-assay variation was approximately 10–15%. Immunoprecipitations and immunoblotting were performed as described (23, 24). Dissolved nonfat dry milk was used to block nonspecific binding sites on nitrocellulose filters.

RESULTS

The distribution of apoE4 and E3 among various plasma lipoproteins in a subject with the E4/3 phenotype was examined by agarose chromatography of plasma samples. Separation of lipoprotein classes by chromatography rather than ultracentrifugation (25) prevented the loss of apoE from the lipoprotein particles. The column fractions containing the VLDL, LDL, and HDL were identified on the basis of the cholesterol distribution pattern (**Fig. 1**). The three pools (I–III) taken corresponded to VLDL, intermediate density lipoproteins (IDL) and LDL, and HDL, respectively. The relative amounts of apoE4 and

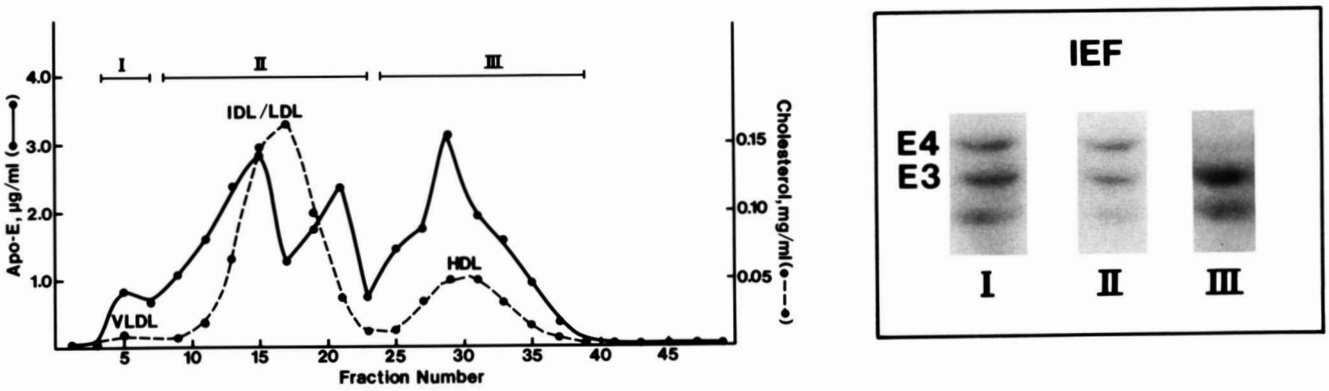

Fig. 1. Distribution of apoE4 and E3 among plasma lipoproteins in a subject with the E4/3 phenotype. Left: Five ml of plasma was chromatographed on a Bio-Gel A-5m column (2.2 × 80 cm) operated at a flow rate of approximately 15 ml/h; after ~90 ml had been eluted from the column, fractions were collected at 15-min intervals. The concentrations of plasma cholesterol and triglyceride in this subject were 226 and 65 mg/dl, respectively. The concentrations of apoE (●—●) in each fraction were determined by solid-phase radioimmunoassay. The cholesterol concentrations (●---●) in each fraction were determined, and three pools (I–III) corresponding to VLDL, IDL/LDL, and HDL, respectively, were taken. Right: Apolipoprotein E-containing lipoproteins were immunoprecipitated and subjected to isoelectric focusing (IEF), and the focused proteins were transferred to nitrocellulose. Isoforms of apoE were detected by using rabbit anti-human apoE and ^{125}I-labeled goat anti-rabbit immunoglobulin G followed by autoradiography.

E3 in the three pools were assessed after immunoprecipitation by isoelectric focusing and immunoblotting. Relative to apoE3, there was more apoE4 in both VLDL (I) and IDL/LDL (II) than in HDL (III) (Fig. 1, inset). This confirms previous results which indicated that apoE4 preferentially distributed with VLDL in subjects with the E4/3 phenotype (14, 15) and demonstrates that the apoE4 preference also extends to the IDL/LDL fraction. These results are also in agreement with the distribution trends in E4/4 and E3/3 homozygotes reported by Gregg et al. (13), who used ultracentrifugation instead of chromatography.

The distribution of a tracer dose of added ^{125}I-labeled apoE3 (i.e., exogenous apoE3) incubated with plasma from an E3/3 subject was compared with the distribution of the subject's own apoE3 (i.e., endogenous apoE3). The exogenous apoE3 was reduced and alkylated with iodoacetamide to prevent cysteine disulfide formation from influencing the result. As shown in **Fig. 2A,** the endogenous and exogenous distributions closely resemble each other, indicating that the exogenous apoE was close to fully equilibrating with the endogenous apoE pool. Identical results were obtained with apoE3 that was only reduced with mercaptoethanol before incubation with plasma (data not shown). Reduction was necessary before incubation because approximately 10–30% of the apoE3 in the preparations used had dimerized via disulfide bond formation during the isolation procedure. Because the distribution of the exogenous ^{125}I-labeled apoE3 mimicked the distribution of the plasma apoE3 associated with the native lipoproteins, similar assay conditions were used to analyze the distribution of ^{125}I-labeled apoE4 in plasma from an apoE3/3 subject. As shown in Fig. 2B, apoE4 accumulated preferentially in the VLDL and large LDL (i.e., IDL), whereas relative to its distribution in VLDL, more apoE3 appears in the HDL. These differ-

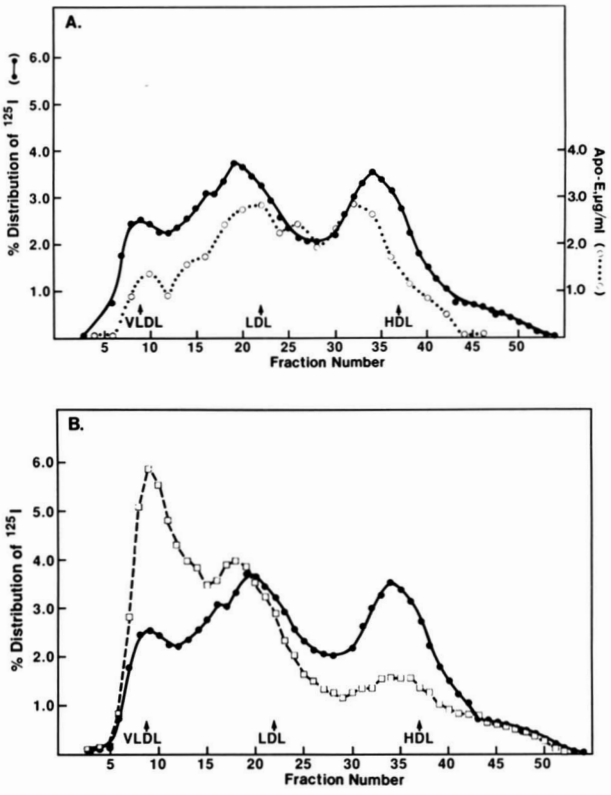

Fig. 2. Distribution of ^{125}I-labeled apoE4 and E3 among plasma lipoproteins. ^{125}I-labeled apoE4 or E3 was incubated with 5 ml of plasma from subject 1 (E3/3 phenotype), and the mixture was subjected to agarose chromatography under the conditions described in the legend to Fig. 1. The concentrations of plasma cholesterol and triglyceride in this subject were 164 and 63 mg/dl, respectively. The percent distribution of cholesterol among the VLDL, IDL/LDL, and HDL fractions was 1.9, 66.4, and 31.7%, respectively. Arrows indicate elution positions of VLDL, IDL/LDL, and HDL; these positions were determined from the cholesterol distribution. A: (○), distribution of endogenous apoE determined by solid-phase radioimmunoassay; (●), distribution of exogenous ^{125}I-labeled apoE3 after incubation with plasma. B: (□), distribution of ^{125}I-labeled apoE4; (●), distribution of ^{125}I-labeled apoE3.

ences in distribution were also observed in four additional apoE3/3 subjects (data not shown). Furthermore, similar distribution differences were obtained when ^{125}I-labeled apoE4 or apoE3 was added to plasma from an apoE4/4 subject (data not shown). These results indicate that the cysteine-arginine interchange at position 112 (which distinguishes E4 from E3) influences the pattern of apoE distribution among lipoproteins. Because the other major polymorphic site on apoE involves a cysteine-arginine interchange at residue 158, it was of interest to determine whether this interchange had a significant effect in addition to that exerted by the interchange at residue 112. When ^{125}I-labeled apoE2 was added to plasma from an apoE3/3 subject, its distribution closely resembled that of apoE3 (**Fig. 3**), suggesting that the cysteine-arginine interchange at residue 158 (which distinguishes E2 from E3) has little additional effect on the distribution.

Because some apoE3 exists in the plasma as the disulfide-linked heterodimer apoE-A-II and this complex has been suggested to account for the distribution of plasma apoE3, it was important to determine how this complex distributed. As shown in **Fig. 4,** apoE3-A-II displayed an even greater preference for HDL than monomeric apoE3 did. Thus, both the formation of apoE3-A-II and an inherent property of the monomeric apoE3 structure related to the cysteine-arginine interchange at position 112 account for the preferential distribution of apoE3 to HDL.

The preceding results demonstrate that the apoE4 preference for VLDL/IDL is determined by the presence of arginine at position 112. Since arginine is a positively charged amino acid, it was of interest to determine whether the apoE4 distribution resulted from the presence of a positive charge at position 112 or specifically from the

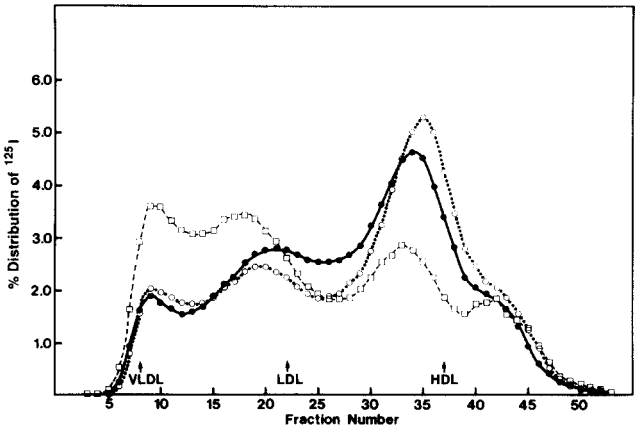

Fig. 3. Distribution of ^{125}I-labeled apoE4, E3, and E2 among plasma lipoproteins. Iodinated apoE was incubated with plasma from subject 1 (E3/3), drawn on a separate occasion from the plasma used for Figs. 2A and B, and chromatographed as described in the legend to Fig. 1; (□), ^{125}I-labeled apoE4; (●), ^{125}I-labeled apoE3; (○), ^{125}I-labeled apoE2; arrows as in Fig. 2.

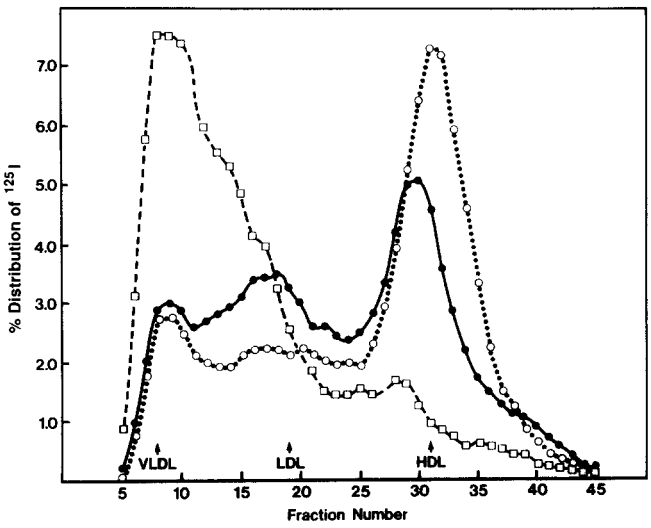

Fig. 4. Distribution of ^{125}I-labeled apoE4, E3, and E-A-II among plasma lipoproteins. Iodinated apoE was incubated with plasma from subject 2 (E3/3) and chromatographed as described in the legend to Fig. 1; (□), ^{125}I-labeled apoE4; (●), ^{125}I-labeled apoE3; (○), ^{125}I-labeled apoE3-A-II; arrows as in Fig. 2. The concentrations of plasma cholesterol and triglyceride were 171 and 89 mg/dl, respectively. The percent distribution of cholesterol among the VLDL, IDL/LDL, and HDL fraction was 3.6, 71.7, and 24.7%, respectively.

presence of arginine at that position. If the distribution was the result of having a positively charged residue at 112, then apoE3 modified with cysteamine, which converts cysteine to a positively charged moiety, would distribute like apoE4. As demonstrated in **Fig. 5,** conversion of cysteine to a lysine analogue as a result of cysteamine modification resulted in an apoE4 distribution pattern. The distribution profiles of apoE4 and cysteamine-modified apoE3 were nearly identical, demonstrating that the presence of a positive charge at residue 112 contributed to the distribution pattern.

The effect of a negative charge at 112 was examined by alkylation of cysteine with iodoacetic acid, which generates a glutamic acid-like side chain. It was found that relative to carboxamidomethylated (iodoacetamide) apoE3, a larger fraction of the carboxymethylated apoE3 was associated with HDL (**Fig. 6**). However, in this case, two apoE peaks were associated with this subject's HDL. The first peak eluted in a position of large HDL and corresponded to the position observed in several other subjects (compare with Figs. 1-5, 7), including this particular subject studied on a separate occasion (see Fig. 8). The second peak eluted with small HDL and corresponded to a shoulder of the first HDL peak in the previous cases. The basis for this double peak within the HDL fraction is not clear, but may be associated with this subject beginning a physical training program since he was last examined. The distribution of the carboxamidomethylated and carboxymethylated apoE3 between HDL and VLDL/IDL/LDL was compared by dividing the profile at frac-

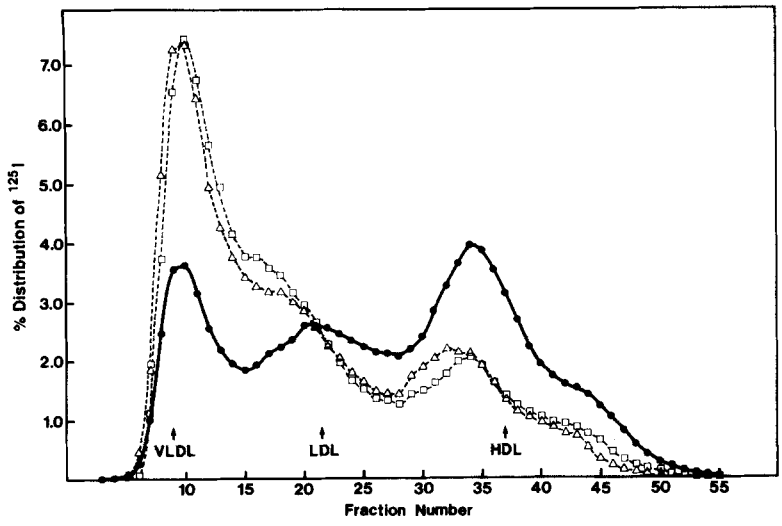

Fig. 5. Distribution of ^{125}I-labeled apoE4, cysteamine-modified E3, and E3 among plasma lipoproteins. Iodinated apoE was incubated with plasma from subject 1 (E3/3) drawn on a third occasion and chromatographed as described in the legend to Fig. 1. The concentrations of plasma cholesterol and triglyceride were 140 and 131 mg/dl, respectively; (□), ^{125}I-labeled apoE4; (△), ^{125}I-labeled apoE3 modified with cysteamine; (●), ^{125}I-labeled apoE3; arrows as in Fig. 2.

tion number 22. With carboxamidomethylated apoE3, 28.1% and 71.9% of the label were associated with VLDL/IDL/LDL and HDL, respectively, while carboxymethylated apoE3 had a distribution split of 16.8% and 83.2%. The distribution of reduced apoE3 more closely resembled the distribution of the carboxamidomethylated apoE3 (not shown). Thus, as long as the residue at position 112 is either neutral or has a negative charge, there is a preferential apoE3 distribution to HDL. However, a negative charge at this position appears to result in a slight additional shift from VLDL/IDL/LDL to HDL.

Physical-chemical studies have demonstrated that in aqueous solution apoE is composed of two independently folded domains and that two thrombolytic fragments of the protein, the 22-kDa (residues 1–191) and the 10-kDa (216–299) fragments, approximate the two domains in many important respects (24, 26). Because the cysteine-arginine interchange at position 112, which distinguishes apoE4 from E3, is located in the amino-terminal domain, it was of interest to determine whether the amino-terminal 22-kDa thrombolytic fragments of apoE4 and E3 distributed like the intact proteins. However, as shown in **Fig. 7,** the apoE3 22-kDa fragment eluted in a lipoprotein-deficient region of the column, indicating that it did not bind to any of the major lipoprotein classes. Moreover, the distribution curves of both the apoE4 and E2 22-kDa fragments were identical to the curve of the apoE3 22-kDa fragment (data not shown). This demonstrates that the amino-terminal domain of apoE does not bind to lipoprotein particles and that the distribution of this fragment is not influenced by the arginine substitution at either position 112 or 158. These results are consistent with the major lipid-binding function of apoE residing in the carboxyl-terminal domain (residues 192–299), as previously suggested (6).

Because the carboxyl-terminal domain was the major lipid-binding region, it was of interest to determine how its distribution compared with that of intact apoE4 and E3. As shown in **Fig. 8,** incubation of the 12-kDa fragment of apoE (residues 192–299) with plasma resulted in a distribution that was different from either the apoE4 or E3 distribution. This indicates that the nature of the amino acid at position 112 in the amino-terminal domain determines which lipoprotein particle the carboxyl-terminal domain binds to.

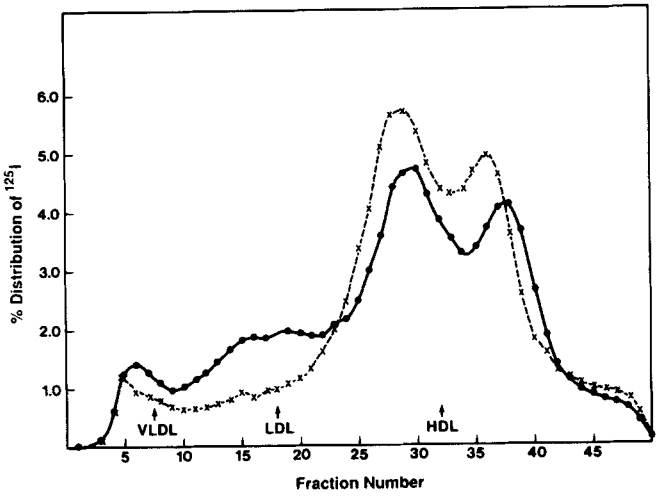

Fig. 6. Distribution among plasma lipoproteins of ^{125}I-labeled carboxamidomethylated and carboxymethylated apoE3. Iodinated apoE was incubated with plasma from subject 3 (E3/3) and chromatographed as described in the legend to Fig. 1; arrows as in Fig. 2. The plasma cholesterol and triglyceride concentrations were 176 and 66 mg/dl, respectively. The percent distribution of cholesterol among the VLDL, IDL/LDL, and HDL fractions was 1.5, 68.5, and 30.0%, respectively; (X), ^{125}I-labeled carboxamidomethylated apoE3; (●), ^{125}I-labeled carboxymethylated apoE3.

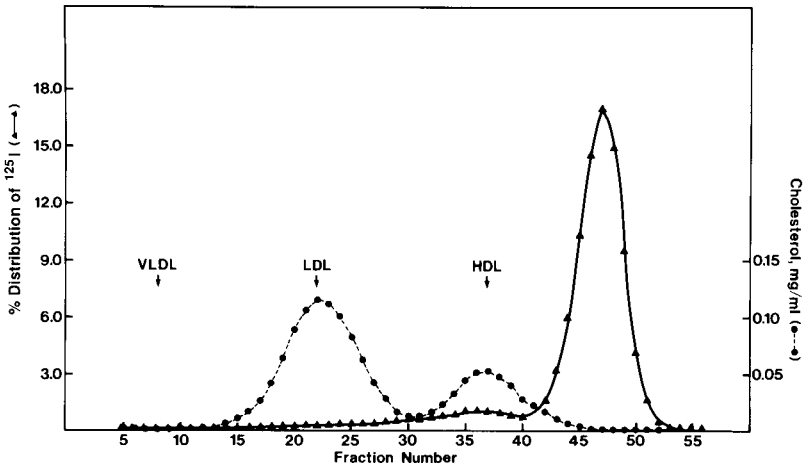

Fig. 7. Distribution of ^{125}I-labeled 22-kDa fragment of apoE3 among plasma lipoproteins. Iodinated apoE3 22-kDa fragments were incubated with plasma from subject 1 (E3/3) drawn on a third occasion and chromatographed as described in the legend to Fig. 1. The concentrations of plasma cholesterol and triglyceride were 146 and 63 mg/dl, respectively; (▲), ^{125}I-labeled apoE3 22-kDa fragment; (●), cholesterol distribution; arrows as in Fig. 2.

DISCUSSION

Examination of the distribution of an E4/3 subject's apoE4 and E3 among plasma lipoproteins separated by agarose column chromatography demonstrated that the isoforms distributed differently. This corroborates and extends previous experiments by Gregg et al. (13) with E4/4 and E3/3 homozygotes, in which ultracentrifugation was used to separate lipoprotein classes. The fact that apoE4 and E3 distribute to separate lipoprotein classes in the same subject rules out the possibility that the differences in distribution observed between E4/4 and E3/3 homozygotes (13) might be due to variations in the lipoproteins of individuals with different phenotypes. The distribution pattern observed in the present study is also consistent with recent reports that compared the distributions of apoE4 and E3 in VLDL and HDL isolated by agarose chromatography from the plasma of E4/3 subjects (14, 15). In the present study, the comparison was extended to include the IDL/LDL fraction; relative to apoE3, there was more apoE4 in both VLDL and IDL/LDL than in HDL.

It is generally assumed that the differences in distribution among lipoprotein classes may be central in determining the different effects that apoE4 and E3 have on plasma cholesterol and LDL concentrations. The preferential distribution of apoE4 to triglyceride-rich lipoproteins (chylomicrons and VLDL) is thought to result in the more rapid uptake of intestinal and hepatic remnant particles because more apoE molecules per particle would give the particle a higher affinity for the LDL receptor. The resulting increased uptake by hepatic receptors would cause their down-regulation (for a more complete discussion, see ref. 4). Consistent with this model is the study of Weintraub, Eisenberg, and Breslow (16), which demonstrated that [^{3}H]retinol-labeled chylomicrons were cleared faster in E4/3 subjects than in E3/3 subjects. These results suggest that there might be a kinetic effect associated with down-regulation of hepatic LDL receptors. It has also been suggested that the accumulation of apoE4 in VLDL facilitates an apoE-mediated conversion

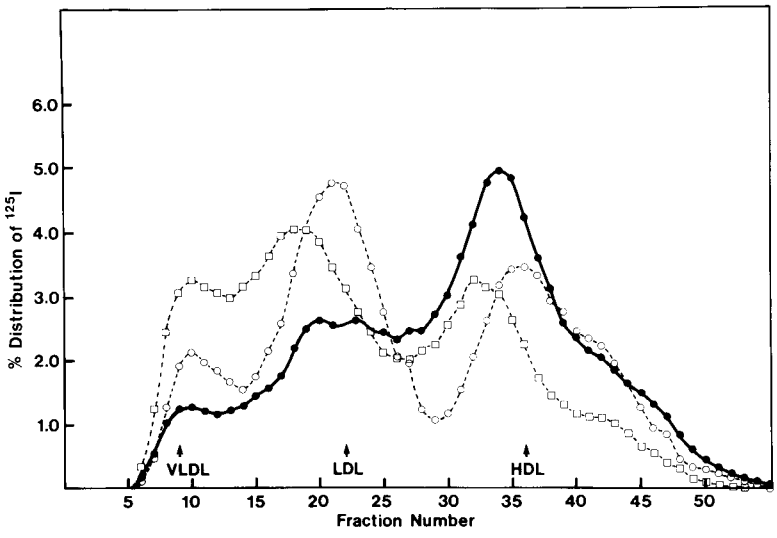

Fig. 8. Distribution of ^{125}I-labeled apoE4, E3, and 12-kDa fragment of apoE3 among plasma lipoproteins. Iodinated apoE and 12-kDa fragments were incubated with plasma from subject 3 (E3/3) drawn on a separate occasion from that described in Fig. 6 and chromatographed as described in the legend to Fig. 1. The concentrations of plasma cholesterol and triglyceride were 177 and 71 mg/dl, respectively. The percent distribution among the VLDL, IDL/LDL, and HDL fractions was 6.1 67.2, and 26.6%, respectively; (□), ^{125}I-labeled apoE4; (●), ^{125}I-labeled apoE3; (○), ^{125}I-labeled 12-kDa fragment; arrows as in Fig. 2.

of the VLDL to LDL (4). This would also increase plasma concentrations of LDL, although there are no experimental data to support this suggestion. In the present study, the IDL fraction (also enriched with apoE4) could also either participate in increased hepatic uptake of IDL or possibly facilitate their conversion to LDL.

Previously, it had been speculated that the apoE3 versus apoE4 distribution was the result of either the presence of arginine at position 112 (apoE4) or the formation of E3–A-II because of the presence of cysteine at 112 in apoE3. This study was designed to address this issue. The structural properties—specifically the presence of arginine versus cysteine at position 112 and the ability of apoE3 to dimerize with apoE–A-II—were examined in an in vitro assay in which ^{125}I-labeled apoE or apoE fragments were incubated with plasma and the distribution of labeled apoE among lipoprotein classes was determined by agarose chromatography. This assay system was designed to focus on the biochemical (structural) parameters and to eliminate metabolic considerations. The single cysteine residue at position 112 in apoE3 was alkylated with iodoacetamide to prevent disulfide bond formation during incubation. Exogenous ^{125}I-labeled apoE3 distributed like endogenous apoE in plasma lipoproteins from a subject with the E3/3 phenotype. A consistent distribution of apoE4 to the VLDL and IDL/LDL fractions and of apoE3 to the HDL fraction was observed in several subjects, regardless of the phenotype of the subjects from whom plasma was obtained for incubation. These results demonstrate that the cysteine-arginine interchange at position 112, which distinguishes apoE4 from E3, is responsible for the distribution differences observed in the iodinated apoE incubations. The distribution of apoE2 was similar to that of apoE3, indicating that the cysteine-arginine interchange at position 158 does not affect distribution.

The heterodimer apoE3–A-II also distributed preferentially to HDL. Thus, the distribution of endogenous apoE3 in E4/3 or E3/3 plasma, in which a portion of apoE3 exists as apoE3–A-II, is the result of a combination of an inherent property of monomeric apoE3 and the formation of apoE3–A-II. Thus, both mechanisms postulated to explain the distribution of apoE3 and E4 contribute to the distribution pattern. Because both apoE3 forms distribute preferentially to HDL, changes in the ratio of monomeric apoE3 to heterodimer are likely to have only minor effects on apoE3 distribution. However, the ratio could have significant effects on lipoprotein metabolism. It has been suggested that the addition of apoA-II might modulate the binding activity of apoE in vivo (27). This modulation of binding activity could account for some of the metabolic differences observed between apoE4 and E3. The ability of apoE to facilitate conversion of IDL to LDL might also account for some of these differences.

Cysteamine modification of the cysteine residue at position 112 of apoE3 results in an apoE4 distribution pattern. This demonstrates that the charge at position 112 is the determining factor in distribution, not the presence of arginine at this site. This finding parallels the preliminary results of Gregg et al. (28): aminoethylation of apoE2 increased the fractional catabolic rate of apoE2 almost to the rate of apoE4. Presumably this modification of apoE2 also resulted in a change in the distribution pattern. In contrast, when the cysteine at position 112 was left unmodified or was modified with iodoacetamide or iodoacetic acid to give either a glutamine-like residue (neutral) or glutamic acid-like residue (negative charge), respectively, there was no change in the preferential distribution to HDL, although the addition of negative charge resulted in a further increase in the label associated with HDL. Thus, it appears likely that a variety of amino acid side chains not involving a positive charge will result in an apoE3 pattern. Further studies will determine whether amino acid substitutions in apoE at sites other than 112 also affect distribution.

Studies on the thrombolytic 22-kDa (residues 1–191) and 12-kDa (192–299) fragments support the suggestion that the 12-kDa fragment, representing the carboxyl-terminal domain of apoE, is the major lipid-binding region of the protein (6). Although the 22-kDa fragment, representing the amino-terminal domain and containing the receptor-binding function, does form complexes with the phospholipid dimyristoylphosphatidylcholine (20), it does not recombine with any of the major lipoprotein classes when incubated with plasma. This is consistent with observations made by Gianturco et al. (29) with regard to thrombolytic digests of VLDL from hypertriglyceridemic subjects. These investigators found that a fraction of apoE on the hypertriglyceridemic VLDL was cleaved by thrombin and that after centrifugation the 22-kDa fragment was lost from the particle, whereas the 12-kDa fragment remained associated. Presumably the presence of several amphipathic α-helices in the 12-kDa fragment account for the strong lipid-binding properties (6).

In aqueous solution apoE contains two independently folded domains whose biochemical make-up and behavior are approximated by the 22- and 10-kDa fragments (24, 26). While there appears to be little interaction between the domains in the absence of lipid (26), results from the present study imply that there is an interaction between the domains when the protein is associated with lipid. Clearly, the cysteine-arginine interchange at position 112 in the amino-terminal domain determines which lipoprotein particle the carboxyl-terminal domain will bind to. Because the two domains appear to be independently folded in aqueous solution, communication or cooperation between domains might suggest a reorganization of protein structure once the protein is on a lipid surface.

The fact that a positive charge at position 112 is critical in determining the distribution implies that a salt bridge might be involved in the interaction. It is possible that the surface composition of the lipoprotein particle, and perhaps the size of the particle or its surface curvature, might play a role in determining how the domains interact. An indication of reorganization has been provided by preliminary results from limited proteolysis of apoE in recombinant phospholipid particles and VLDL particles. In contrast to the findings in aqueous solution, where the inter-domain region of residues 166–224 was highly susceptible to proteolysis, this region becomes resistant to cleavage by a wide variety of proteases when apoE is combined with lipid. Modulation of the function in one structural domain by the second domain in the presence of lipid has been observed previously in apoE (30). In that case, the carboxyl-terminal domain of apoE2 modulated receptor-binding activity in the amino-terminal domain.

In summary, it has been demonstrated that the cysteine-arginine interchange at position 112 in apoE determines which lipoproteins apoE4 and E3 will bind to. The determining factor in the preferential distribution of apoE4 to VLDL and IDL/LDL is a positive charge at position 112. The introduction of neutral or negatively charged side chains to the cysteine at position 112 in apoE3 does not significantly influence its preferential distribution to HDL. In addition, the heterodimer apoE3-A-II also distributes primarily to HDL. Thus, both the monomeric and heterodimeric forms of apoE3 contribute to the apoE3 distribution observed in plasma. The results of experiments with apoE fragments indicate an interaction between the two structural domains of apoE; the amino acid residue at position 112 in the amino-terminal domain determines which lipoprotein particle the carboxyl-terminal domain binds to.

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