Isolation of subpopulations of high density lipoproteins: three particle species containing apoE and two species devoid of apoE that have affinity for heparin

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Abstract We have isolated and partially characterized five populations of lipoproteins from the pool of immunoisolated apoA-I-containing lipoproteins obtained from normal human plasma. The first three populations, each containing apoA-I and apoE, were isolated completely by sequential, selected affinity immunosorption against apoA-I and apoE. The lipoproteins isolated by this strategy fall into three morphologic groups; there are discs (LP-AI-E(1)), small spherical lipoproteins (LP-AI-E(2)), and large spherical lipoproteins (LP-AI-E(3)). The LP-AI-E(2) species was sufficiently abundant for detailed characterization. They have slightly larger diameters, and contain more lipid than the bulk of apoA-I-containing lipoproteins and they contain apoA-II:E heterodimers and apoE homodimers. Core lipids are enriched in triglyceride relative to cholesteryl esters. These lipoproteins compete with LDL equally, on a protein mass basis, for binding to human fibroblasts. After removal of apoE-containing lipoproteins from the pool of apoA-I-containing lipoproteins, we discovered two additional subpopulations of lipoproteins that bind to heparin. These lipoproteins, devoid of apoE, occur as populations of small, (LP-AI-HB(1)), and large, spherical lipoproteins, (LP-AI-HB(2). The heparin-binding lipoproteins were separated by gel permeation chromatography. The LP-AI-HB(1) population was of sufficient quantity for detailed study. These lipoproteins also had larger diameters than the bulk of HDL but their core lipids were enriched in cholesteryl esters rather than triglycerides. Three proteins associated with these lipoproteins were found to bind to heparin-Sepharose in the absence of lipid. The approximate molecular weights of these proteins are 40, 70, and 90 kDa. The 70 kDa molecule was found to be the SP 40,40 protein (apoJ).—**Hennessey, L. K., S. T. Kunitake, M. Jarvis, R. L. Hamilton, G. Endeman, A. Protter, and J. P. Kane.** Isolation of subpopulations of high density lipoproteins: three particle species containing apoE and two species devoid of apoE that have affinity for heparin. *J. Lipid Res.* 1997. **38:** 1859–1868.

Supplementary key words molecular species • immunosorption • receptor binding • electron microscopy • apolipoprotein J

Plasma high density lipoproteins (HDL) are not homogeneous (1–5). When prepared by techniques that exclude ultracentrifugation, native HDL comprise a number of discrete species varying in their lipid and protein stoichiometries. Such speciation is obscured when ultracentrifugation is used because high pressure during ultracentrifugation dissociates apolipoproteins from HDL complexes and favors the formation of abnormal recombinant particles (6–10). A common feature of all lipoproteins functionally defined as HDL is that they contain apolipoprotein (apo) A-I. These apoA-I-containing lipoproteins can be subfractionated on the basis of the other apolipoproteins that are present on individual particles. Because apolipoproteins appear to be connected with biological function, a logical approach to the separation of HDL subspecies can be based on the individual apolipoproteins that are present. This approach has been used to separate HDL into several particle populations.

For example, using secondary immunosorption, HDL have been separated into subpopulations containing apoA-II and devoid of apoA-II (11–17). Functional differences in these two subpopulations have been observed with respect to binding to cell surfaces (15, 18), efflux of cellular cholesterol (19–21), and particle degradation (22, 23). Conversely, both subfractions have equal ability to generate prebeta HDL when cholesteryl esters are transferred from HDL to lipoprotein acceptors by cholesteryl ester transfer protein (24). Discrete HDL species containing transferrin and ceruloplasmin, respectively (25), and apolipoprotein A-IV (26), have also been isolated.

Abbreviations: apo, apolipoprotein; LP-AI-E, lipoproteins containing apoA-I and apoE; LP-AI-HB, apoA-I-containing lipoproteins that bind to heparin.

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A potentially interesting functional subpopulation of HDL are those lipoproteins that also contain apoE (LP-AI-E). ApoE is a ligand for both the LDL receptor (27) and the α2-macroglobulin receptor (28). Furthermore, it has been reported that over half the circulating apoE resides on HDL (29, 30) and that these apoE-containing HDL do appear to interact with the LDL receptor (31). In addition, a recent study has suggested that the inverse relationship between HDL and coronary risk is attributable, at least in part, to apoE-containing HDL (32).

ApoE-containing HDL have been isolated (4, 32–36), but their isolation has generally involved a combination of sequential ultracentrifugation and heparin-Sepharose chromatography. Sequential ultracentrifugation has been documented to cause the dissociation of apolipoproteins from lipoproteins, altering their native structures (6–10). Specifically, it has been shown that apoE is dislodged from VLDL by ultracentrifugation and transferred to HDL or recovered in a lipoprotein-free dispersed state (37). Such a redistribution of apoE could lead to unphysiologic apoE-HDL complexes that might have abnormal biological activities. Heparin-Sepharose chromatography is thought to retain those lipoproteins containing either apoB or apoE (35) exclusively. However, immunoisolated lipoproteins could contain other proteins capable of interacting with heparin.

In this study we report the isolation and partial characterization of two subpopulations of apoA-I-containing lipoproteins isolated by the minimally perturbing method of selected affinity immunosorption. The first subpopulation that contains apoE as well as apoA-I (LP-AI-E) was isolated with an anti-apoE Sepharose column from the total HDL pool. The second subpopulation of HDL was found to bind to heparin-Sepharose (LP-AI-HB) but was devoid of apoE.

METHODS

Isolation of lipoproteins

Venous blood was drawn from fasting, normolipidemic, apoE 3/3 homozygotic subjects (2 male and 1 female) and immediately mixed with the preservatives, ethylene-diaminetetraacetic acid (EDTA, 0.08% W/V), sodium azide (0.1% W/V), benzamidine (500 μg/ml), phenylmethanesulfonyl fluoride (10 μg/ml), ϵ aminocaproic acid (3 mg/ml), and gentamycin sulfate (10 μg/ml) (final concentrations). Plasma was separated by centrifugation at 1000 *g* for 45 min at 4°C and stored at 4°C for up to 1 week. α 2-Macroglobulin (10 μg/ml) and the above-listed preservatives were added at all steps in lipoprotein isolation and to all isolated lipoproteins.

Apolipoprotein A-I-containing lipoproteins (HDL) were isolated by selected affinity immunosorption (38, 39), as previously described. Briefly, plasma was applied to an immunoaffinity column made with antibodies directed against human apoA-I and pre-selected for their ability to dissociate from apoA-I under the chosen elution conditions. Unbound proteins were washed from the column with 150 mM NaCl, 5 mM Tris, pH 7.4 (TBS). Subsequently, all of the bound lipoproteins were eluted with 0.2 M acetic acid, pH 3.0. The eluate was neutralized immediately with 2 M Tris, concentrated under nitrogen (20 psi) using an Amicon ultrafiltration cell fitted with a YM 10 membrane (Danvers, MA), and exchanged into TBS. The selected affinity immunosorption columns provide a recovery of 98.5%, by ELISA assay, of the apoA-I applied. The eluate is then passed through protein A-Sepharose and anti-human albumin-Sepharose columns for removal of trace contamination with immunoglobulins or albumin. The absence of lipid in the proteins sorbed to the two latter columns indicates that the albumin and immunoglobulin removed are merely residual traces of these proteins which are very abundant in plasma and do not reflect HDL species in which they might be integral constituents.

Lipoproteins containing apoA-I and apoE, (LP-AI-E) were isolated by a second round of selected affinity immunosorption analogous to the method described above (Fig. 1). Antibodies directed against human apoE were isolated by the passage of rabbit antiserum through an apoE-Sepharose column. The bound antibodies were eluted with 0.2 M acetic acid, pH 3.0. The purity of the apoE used as the antigen and for the construction of the apoE-Sepharose column was confirmed by SDS-PAGE and Western blotting (data not shown). The selected antibodies were then used to construct an anti-apoE-Sepharose column. LP-AI was applied to the column and unbound lipoproteins were washed out with greater than ten column volumes of TBS. The LP-AI-E were recovered by eluting the column with 0.2 M acetic acid, pH 3.0. The eluate was then processed as described above. ApoE comprised less than 0.2% of the total protein mass of HDL that failed to bind to the apoE immunoaffinity column.

ApoA-I-containing lipoproteins that bind heparin (LP-AI-HB) were isolated by heparin-Sepharose chromatography (Fig. 1) (36). Briefly, after removal of the apoE-containing particles, 10 mM $MnCl_2$ (final concentration) was added to an aliquot of the lipoproteins and applied to a heparin-Sepharose column (Pharmacia, Uppsala, Sweden). The column was washed with 0.05 M NaCl, 0.005 M Tris, 10 mM $MnCl_2$, pH 7.4 (low salt buffer) until all the unbound lipoproteins were cleared. Then the bound lipoproteins (LP-AI-HB) were eluted with 0.5 M NaCl, 0.05 M Tris, pH 7.4 (high salt buffer),

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concentrated as described above, and equilbrated with TBS containing preservatives.

Both LP-AI-E and LP-AI-HB were fractionated further by gel permeation chromatography with a Pharmacia Superose 12 column (1 × 30 cm). The elution buffer (TBS) was pumped at a flow rate of 0.4 ml/min. The elution of the lipoproteins was monitored by measuring the absorbance of the effluent at 280 nm. The lipoproteins were collected in 0.2-ml fractions. The peak fractions were combined and concentrated as described above.

LDL were isolated by sequential ultracentrifugation (40). The lipoproteins were isolated between the density limits 1.019–1.063 gm/ml with the non-protein solvent density adjusted by the addition of anhydrous KBr. Centrifugation was performed using a Beckman preparative ultracentrifuge and a 60 Ti rotor with run conditions of 50,000 rpm, 22 h, and 4°C. To insure purity, centrifugation was repeated at each density limit.

Analysis of isolated lipoproteins

The chemical composition of the isolated lipoproteins was determined. The protein content of the lipoproteins was determined by the method of Lowry et al. (41) using a protein standard (Pierce, Rockford, IL), which was calibrated against the Center for Disease Control reference standard. Cholesterol and cholesteryl ester were measured by a fluorometric enzymatic method (42), phospholipid by measurement of the lipid-associated phosphorus content (43), and triglycerides by enzymatic determination of the glycerol moiety (Sigma, St. Louis, MO) (44).

Particle sizes were determined from electron micrographs using a computer-based method developed in this laboratory (45). The lipoproteins were negatively stained with 2% potassium phosphotungstate and photographed with a Siemens 101 electron microscope. Final magnification was 1:180,000. The radii of 200 individual free-standing particles were measured by computer-linked digitization according to a standard sampling algorithm. Particle sizes were confirmed by nondenaturing gradient gel electrophoresis (46) and gel permeation chromatography (Pharmacia, Uppsala) using a Pharmacia Superose 12 column.

The proteins found on the isolated lipoproteins were analyzed by electrophoresis in 0.1% sodium dodecyl sulfate (SDS-PAGE) on a 5–25% polyacrylamide gradient gel (47). After electrophoresis the gels were stained with Coomassie Blue R-250 or transferred onto nitrocellulose sheets (48) for Western blotting.

The electrophoretic mobilities of the isolated subfractions were determined by agarose electrophoresis and by immunoelectrophoresis in agarose. Agarose electrophoresis was performed as previously described (49) except that the agarose strips were stained with a solution of 0.2% Amido Black in water–methanol–acetic acid 5:5:1. Immunoelectrophoresis was performed as described by Grabar and Williams (50) using antisera directed against apoA-I (Tago, Burlingame, CA).

Isolation of heparin-binding proteins from LP-AI-HB lipoproteins

LP-AI-HB particles were delipidated and their proteins were precipitated by extraction of one volume of lipoprotein solution in 20 volumes of ethanol–diethyl ether 3:1 for 24 h at −20°C. After washing the precipitated protein once with diethyl ether, the proteins were resolubilzed with 6 M cyanate-free urea. The proteins were then dialyzed into low salt buffer (0.05 M). The proteins were applied to a heparin-Sepharose column, and unbound proteins were eluted with low salt buffer. The bound proteins were then eluted with the high salt buffer. The recovered proteins were concentrated and exchanged into TBS as described above. They were analyzed by SDS-PAGE and Western blotting. Their amino-terminal sequences were determined by Edman chemistry as described by Hunkapillar et al. (51).

Binding studies of LP-AI-E and HDL-HB

Human foreskin fibroblasts (Hs68) from ATCC were cultured in Dulbecco's modified Eagle's medium (DMEM) with 4.5 g/L glucose and 10% fetal bovine serum (Gibco). Eighty % confluent monolayers in 24-well plates were washed 3 times with DMEM and incubated with DMEM with lipoprotein-deficient serum (5 mg/ml protein) for 48 h in order to up-regulate expression of the LDL receptor. LDL, HDL, LP-AI-E, and LP-AI-HB were labeled with ^{125}I by the iodine monochloride method (52) as described (53). Binding studies were carried out for 2 h at 0°C, in DMEM, plus 20 mM Hepco, pH 7.4, and 1 mg/ml fatty acid– and immunoglobulin-free bovine serum albumin, without bicarbonate. This was followed by washing with cold phosphate-buffered saline with 1 mg/ml bovine albumin, containing 0.9 mM $CaCl_2$ and 0.5 mM $MgCl_2$, and twice with PBS alone. Bound ligand was counted after solubilization for 1 h at 37°C in 0.2 N NaOH.

RESULTS

We have isolated lipoproteins containing both apoA-I and apoE (LP-AI-E) from E 3/3 homozygous subjects completely by selected affinity immunosorption (**Fig. 1**). First, the LP-AI were isolated from plasma and then the apoE-containing lipoproteins were isolated from the total pool of LP-AI. Approximately 60% of apoE in plasma was found associated with LP-AI. We examined the apoA-I particles that were not bound by the anti-

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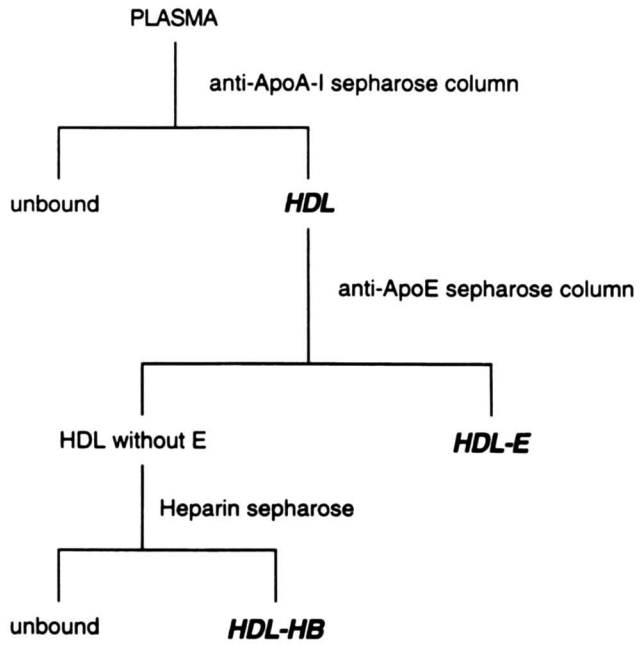

Fig. 1. Diagram of the sequential selective affinity immunosorption steps used to isolate HDL, HDL-E, and HDL-HB subfractions from plasma.

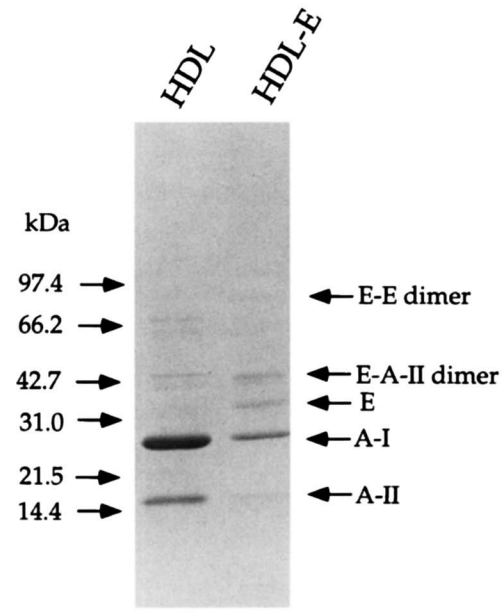

Fig. 2. Analysis of HDL and HDL-E subfractions. Twenty µg of total protein from each fraction were electrophoresed on a 5–25% SDS-PAGE gel and then stained with Coomassie blue.

apoE affinity column to determine whether the binding was complete. We found that apoE comprised only 0.2% of the protein mass of the unbound fraction, indicating that the binding to the columns was virtually quantitative. The LP-AI-E had alpha migration in agarose gel electrophoresis.

Chemical analysis of LP-AI-E revealed that this subfraction was strikingly enriched in triglyceride compared to the bulk of apoA-I-containing particles (**Table 1**). The proteins associated with LP-AI-E particles were analyzed by SDS-PAGE (**Fig. 2**). In addition to apoA-I and apoA-II, a number of Coomassie-staining bands were present. Three protein bands at approximately 35, 42, and 90 kDa appear to be enhanced in the LP-AI-E subfraction compared to unfractionated apoA-I-containing lipoproteins. Western blotting with antisera specific for apoE and apoA-II indicated that these bands were the apoE monomer, E-AII dimer, and E-E dimer, respectively (**Fig. 3**). Reduction of the samples prior to immunoblotting with apoE and apoA-II confirmed the presence of these proteins on LP-AI-E. Due to the presence of some very large spherical particles, the apoE-containing fraction was also tested for apoB. ApoB was detected in this fraction by Western blotting but was an extremely minor component (not visible by Coomassie blue stain).

When the LP-AI-E were viewed by electron microscopy they were found to distribute among three basic types of particles: disks (LP-AI-E(1)), small spherical lipoproteins (particle diameters approximately 10.0 nm) (LP-AI-E(2)), and large spherical lipoproteins (particle diameters greater than 40.0 nm) (LP-AI-E(3)), (**Fig. 4**). These lipoproteins could be separated into two populations by gel permeation chromatography using a Superose 12 column. The peak eluting at the void volume contained the large spherical lipoproteins, disks, and very few of the small spherical lipoproteins. The peak eluting within the included volume chiefly contained small spherical lipoproteins (LP-AI-E (2)).

TABLE 1. Percent chemical composition of LP-A-I, LP-AI-E, and LP-AI-HB

	Protein	Phospholipid	Free Cholesterol	Cholesteryl Ester	Triglyceride
LP-A-I[a]	62.3 ± 1.2	20.9 ± 0.6	1.3 ± 0.1	10.8 ± 0.1	4.7 ± 2.5
LP-AI-E[a]	45.4 ± 1.8	20.4 ± 8.9	4.2 ± 2.0	11.2 ± 4.4	19.0 ± 4.2
LP-AI-E(2)[a]	57.3 ± 5.8	19.9 ± 2.3	1.2 ± 2.3	5.3 ± 0.6	16.4 ± 2.9
LP-AI-HB[b]	55.8 ± 6.2	21.9 ± 0.9	3.5 ± 0.9	11.2 ± 2.1	7.8 ± 3.6
LP-AI-HB(1)[b]	54.0 ± 3.3	25.2 ± 1.6	2.4 ± 0.5	12.9 ± 1.6	5.5 ± 2.8

Results are expressed as mean ± standard deviation.
[a]n = 5 subjects.
[b]n = 4 subjects.

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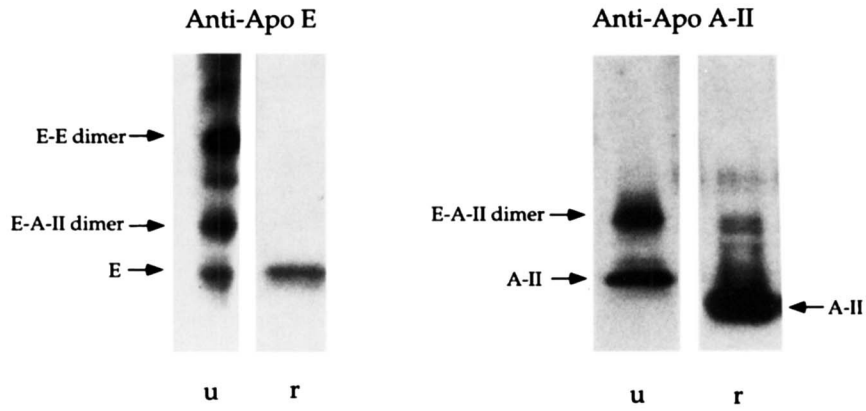

Fig. 3. Western blot of HDL-E. Twenty μg of total HDL-E protein, either unreduced (u) or reduced (r) with beta mercaptoethanol, were resolved on a 5–25% SDS-PAGE gel. Antisera used were against apoE and apoA-II.

Diameters of the small lipoproteins LP-AI-E(2) were obtained from electron micrographs and found to average slightly larger than the average particle diameter of total HDL, 11.2 nm versus 10.0 nm, respectively. As expected, these particles have a higher protein content than the pool of LP-AI-E, but their lipid cores are strikingly higher in triglyceride content relative to cholesteryl ester (Table 1). The apoE content of this fraction was found to be 20% of total protein mass.

The apoE-containing particles were tested for their ability to compete with LDL for binding to cultured fibroblasts (presumably to the LDL receptor). The Lp(A-I-E) were effective competitors of LDL binding, while total LP-AI caused minimal displacement of LDL (**Fig. 5**). The apoE-containing particles were as effective as unlabeled LDL in the displacement of labeled LDL on a protein mass basis. In contrast, the LP-AI-HB subfraction did not displace LDL binding to fibroblasts (data not shown).

Subsequent to the removal of LP-AI-E from the total pool of apoA-I particles, we discovered a subfraction that could bind to heparin-Sepharose (LP-AI-HB).

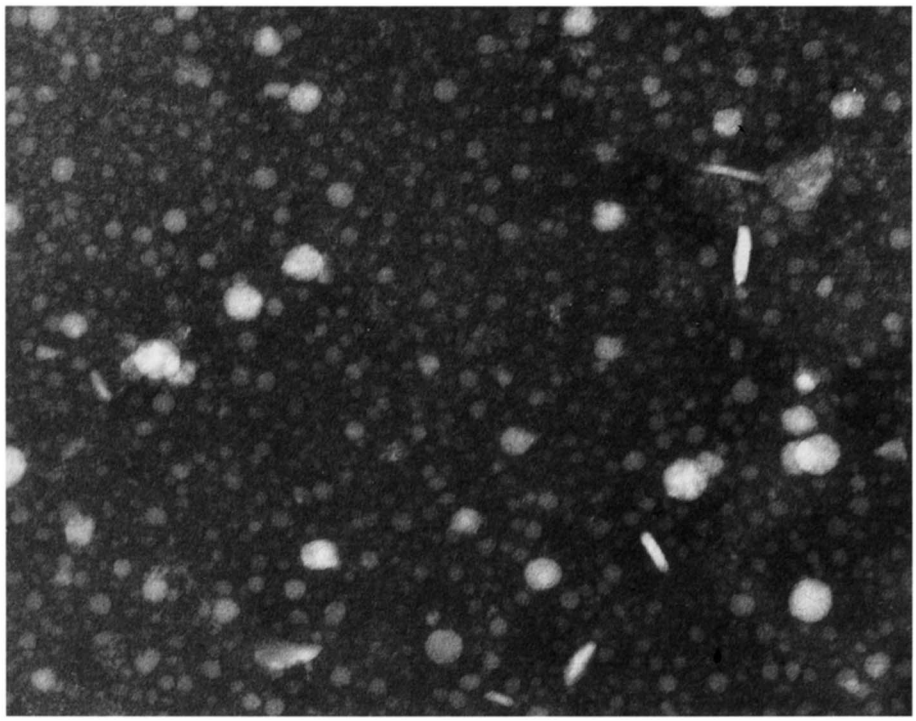

Fig. 4. Elecron micrograph of HDL-E subfraction. HDL-E were negatively stained with phosphotungstate and electron micrographs were taken. The magnification factor is 180,000.

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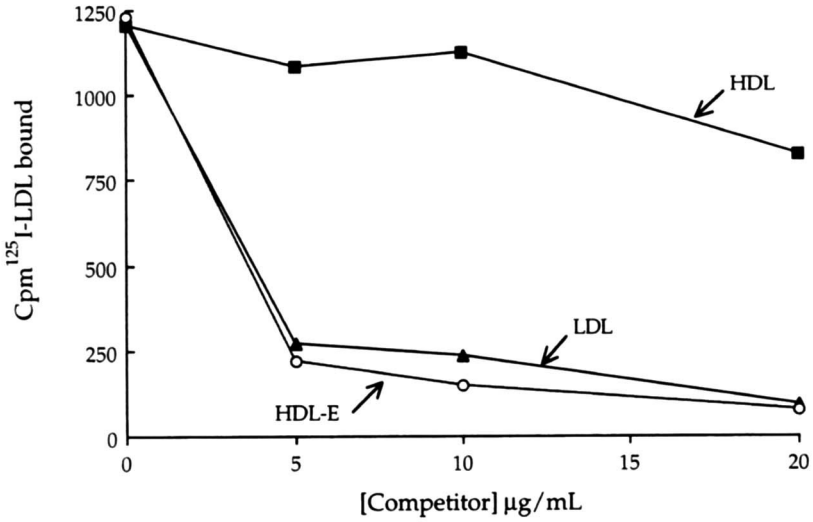

Fig. 5. HDL-E binds the LDL receptor on fibroblasts. Two μg/ml ^{125}I-labeled LDL was incubated with human foreskin fibroblasts for 2 h on ice in the presence of the indicated levels of the unlabeled competitors: LDL, HDL, and HDL-E. These findings are representative of the results obtained from three separate experiments.

These lipoproteins accounted for approximately 20–30% of the mass of apoA-I remaining after the removal of the apoE-containing particles. This lipoprotein subfraction was also more lipid-rich than the total pool of apoA-I-containing particles (Table 1). The LP-AI-HB subfraction showed alpha migration in agarose gels.

The LP-AI-HB were also found to be polydisperse in size when viewed by electron microscopy (**Fig. 6**). There were two types of particles, small spherical lipoproteins (LP-AI-HB(1)) and large spherical lipoproteins, (LP-AI-HB(2)). The LP-AI-HB(1) were separated from the larger HB particles by gel permeation chromatography. The LP-AI-HB(1) particles were found to have a mean diameter slightly larger than that of total apoA-I-containing particles, 11.0 nm compared to 10.0 nm, respectively, by negative staining electron microscopy. This subfraction was found to have a higher lipid content than total LP-A-I as well (Table 1). However, unlike the

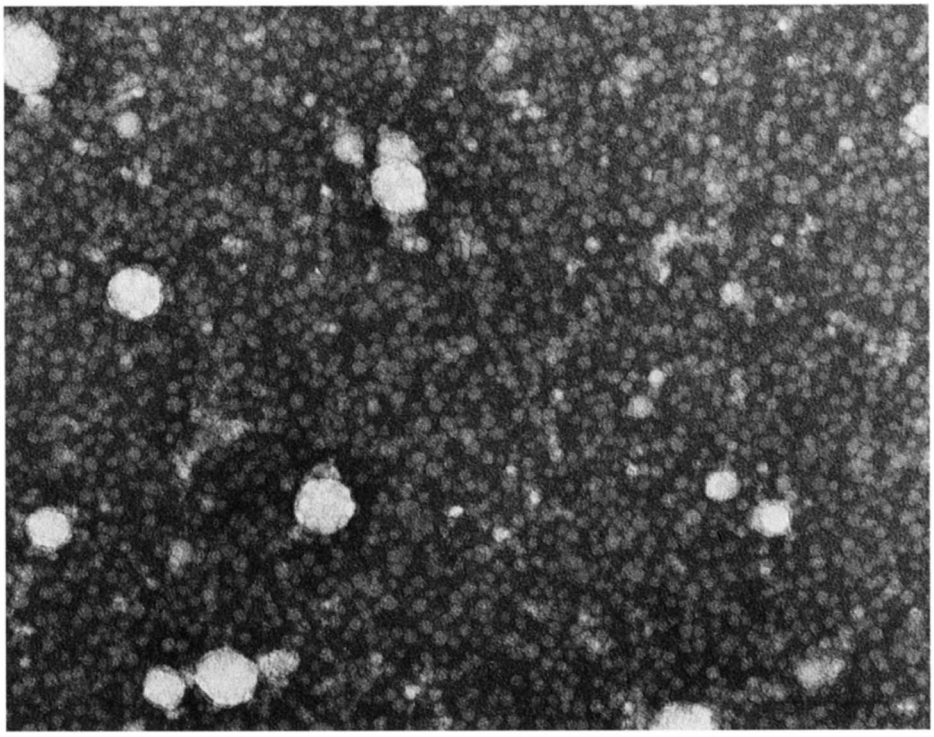

Fig. 6. Electron micrograph of HDL-HB subfraction. HDL-HB were negatively stained with phosphotungstate. The magnification factor is 180,000.

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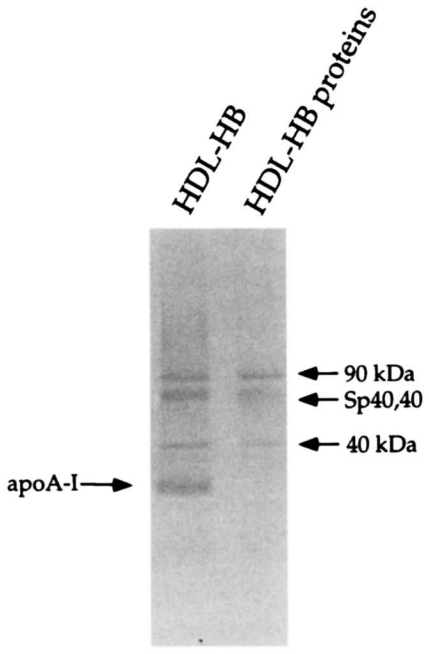

Fig. 7. Coomassie-stained gel of HDL-HB and HDL-HB proteins. The HDL-HB subfraction was delipidated and proteins were resolubilized and repassed over the heparin-Sepharose column. Ten μg of the bound fraction was analyzed on 5–25% SDS-PAGE.

LP-AI-E(2) subfraction, HB(2) subfraction had lipid cores that were enriched in cholesteryl esters instead of triglyceride.

The apolipoprotein contents of the HB subfraction were analyzed by SDS-PAGE (**Fig. 7**). Both apoA-I and apoA-II were identified. No apoE was detected by Western blotting (data not shown). However, three proteins of approximately 90, 70, and 40 kDa appeared to be enriched in this subpopulation compared to the proteins found associated with total LP-A-I. To determine which of the HB proteins was responsible for the binding to heparin-Sepharose, the LP-AI-HB particles were delipidated, resolubilized, and applied to a heparin-Sepharose column. The three delipidated proteins of approximately 90, 70, and 40 kDa were all retained by the heparin-Sepharose column. In contrast, apoA-I (the most abundant protein in LP-AI-HB) was barely detected among the reisolated proteins (Fig. 7). The N-terminal amino acid sequences of these three proteins were determined. The sequences from two of the proteins did not match with the sequence of any protein in the data bank. However, the 70 kDa protein matched perfectly with human SP 40,40 (apolipoprotein J). This identity was confirmed by immunoblotting with focal polyclonal antisera directed against synthetic peptides that were identical to internal sequences of the SP-A and SP-B submits of apoJ, respectively.

DISCUSSION

The exclusive use of selected affinity immunosorption has allowed us to isolate lipoprotein subspecies that contain both apoA-I and apoE. In addition, we have discovered a subpopulation of LP-AI that binds to heparin-Sepharose but that does not contain apoE.

Characterization of the apoE-containing species is likely to contribute to understanding of lipid transport processes at a molecular level. ApoE binds to the LDL receptor (27) and mobilizes cellular lipid (54, 55). In humans, about half of the plasma apoE appears to be associated with HDL (29, 37). The apoE in fasting normolipidemic subjects appears to reside on particles that also contain apoA-I. We found no particles that contain apoE only as is the case in cholesterol-fed animals (10).

Most attempts at isolation and characterization of apoE-containing HDL have involved a combination of sequential ultracentrifugation and heparin-Sepharose chromatography (33, 34, 36). The lipoproteins isolated in this manner have been described as being cholesteryl ester-rich and somewhat larger than the average HDL. They have been reported to bind to the LDL receptor but with a significantly lower binding constant than LDL (31). However, isolation by sequential ultracentrifugation alters the structure of HDL and causes the redistribution of apoE among the lipoprotein fractions, thus obscuring the characterization of the naturally occurring apoE-containing HDL. Two early reports on isolation of E-containing HDL by anti-apoE immunoaffinity chromatography and gel permeation chromatography did not include characterization of the isolated particles (56, 57).

Combining sequentially, selected affinity immunosorption steps directed against apoA-I and apoE, we isolated lipoproteins containing apoA-I and apoE (LP-AI-E) without the use of ultracentrifugation. The small spherical lipoproteins (LP-AI-E(2)) isolated by this method shared some features in common with apoE-containing HDL isolated by a combination of sequential ultracentrifugation and heparin-Sepharose chromatography. The LP-AI-E(2) were slightly larger than average HDL particles. They also contained all three forms of apoE, apoE monomer, E-AII dimer, and E-E dimer, that have been reported in centrifugally isolated apoE-containing HDL. However, there were a number of differences between the particles isolated by the two different methods. The immunoisolated particles were relatively triglyceride-rich instead of being cholesteryl ester-rich. The distribution of apoE forms found in LP-AI-E was different than that found for ultracentrigually isolated HDL. Whereas apoE was a prominent protein of LP-AI-E it was not the major protein as seen in some centrifugally isolated apoE-containing HDL. The apoE content

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of LP-AI-E(2) is approximately 20% of the total protein content, suggesting that there is one copy of apoE per particle except for the particles that contain the E-E dimer. The immunoisolated particles appear to be more effective at competing for binding to the LDL receptor than are the centrifugally isolated apoE-containing HDL, being equivalent on a protein mass basis to apoB for the binding to the receptor. Thus, it is likely that the conformational disposition of apoE is altered during ultracentrifugation.

In this report, we have described the isolation and partial characterization of previously unrecognized subpopulations of apoA-I-containing lipoproteins that are capable of binding to heparin-Sepharose but do not contain apoE (LP-AI-HB). These particles are also somewhat larger in diameter than average HDL; however, in contrast to the apoE-containing particles, they are enriched in cholesteryl esters instead of triglycerides. The particles contain three proteins of approximately 40, 70, and 90 kDa. These proteins do appear to be associated with the apoA-I lipoproteins because they remained associated with the apoA-I HDL particles despite extensive washing of the columns and they were not detected in eluates from sham columns constructed with antibodies from rabbits not immunized to apoA-I. Evidence that these proteins are responsible for the binding of particles to the heparin-Sepharose column is that when the delipidated and resolublized proteins were rerun through the heparin-Sepharose column, the three proteins were again retained, whereas apoA-I was not. Identity of the 70 kDa protein with the SP-40,40 protein (apo J) was established. The two other proteins have not been described previously.

SP-40,40 (apo J) is a component of the SC 5b-9 complement complex (58, 59). It is known to associate with HDL (60–62). Lipoproteins have been isolated by immunoaffinity chromatography with antibodies against apoJ. de Silva et al. (60) observed five groups based on size: 80, 160, 240, 340, and 560 kDa. However, only the 160, 240, and 340 kDa fractions also contained significant amounts of apoA-I. Jenne et al. (61) determined the composition of these complex particles and found that as an aggregate they were composed of 78% protein and that cholesteryl esters were the predominant class of lipids present.

At first glance these findings seem to be inconsistent with our characterization of the Lp-AI-HB but a number of possible explanations can reconcile these results. de Silva (60) found that the bulk of the particles containing both apoJ and apoA-I were in the 240 and 340 kDa fractions, agreeing with the notion that the particles are larger than average HDL particles. They also found that the 80 and 560 kDa fractions were chiefly comprised of protein. This could explain the high protein content Jenne et al. (61) found for the SP-40,40 containing particles. Another explanation is that the apoJ-containing lipoproteins are only a portion of the LP-AI-HB, but as these particles seem fairly homogeneous in size it is unlikely that the apoJ-containing LP-AI-HB would be significantly different from the remaining LP-AI-HB particles.

It is interesting to note that while apoE and the other heparin-binding proteins reside on LP-AI lipoproteins of similar size, the core lipid compositions of these two subfractions differ significantly. It appears that apoE has a preferential affinity for particles with a high triglyceride to cholesteryl ester ratio. It is possible that these subspecies are formed or at least enriched in triglycerides during the CETP-mediated transfer of cholesteryl esters from apoA-I-containing lipoproteins to acceptor lipoproteins. The increased triglyceride content would facilitate binding of apoE, creating a preferential substrate for hepatic lipase.

Until this report it was thought that the only apolipoproteins to interact with heparin were apoB and apoE. As we have now found additional proteins associated with Lp(A-I) that are capable of interacting with heparin, this suggests that several species of HDL may interact with glycosaminoglycans in vivo. The biological significance of the interaction between apoB and apoE and heparin is not understood; however, it has been suggested that this interaction facilitates the endocytosis of apoB-containing lipoproteins during atherogensis (63). The biological significance of HDL binding to heparin is also not readily apparent. Perhaps its function may be to extend the residence time of HDL in places where there is an abundance of glycosaminoglycans, such as atherosclerotic plaques, thereby increasing the acquisition of cholesterol by apoA-I-containing particles. Also, binding to glycosaminoglycans may facilitate the interactions of the particles with hepatic lipase (63).

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