Lipoprotein B37, a Naturally Occurring Lipoprotein Containing the Amino-terminal Portion of Apolipoprotein B100, Does Not Bind to the Apolipoprotein B,E(Low Density Lipoprotein) Receptor*

Stephen G. Young‡‡, Frank P. Peralta¶, Brian W. Dubois¶¶, Linda K. Curtiss***‡‡, Janet K. Boyles¶¶¶, and Joseph L. Witzum†††‡‡‡

From the ‡Cardiology Division, San Diego Veterans Administration Hospital and the University of California, San Diego, La Jolla, California 92037, §Endocrinology/Metabolism Division, University of California, San Diego, La Jolla, California 92037, **Department of Immunology, Research Institute of Scripps Clinic, La Jolla, California 92037, and §§Gladstone Foundation Laboratories, Cardiovascular Research Institute, Department of Pathology, University of California, San Francisco, California 94140-0608

In 1979, Steinberg and colleagues described a unique kindred with familial hypobetalipoproteinemia (Steinberg, D., Grundy, S. M., Mok, H. Y. I., Turner, J. D., Weinstein, D. B., Brown, W. V., and Albers, J. J. [1979] J. Clin. Invest. 64, 292–301). Recently, we demonstrated the existence of an abnormal species of apolipoprotein (apo-) B, apo-B37 (M, ~203,000), in nine members of that kindred (Young, S. G., Bertics, S. J., Curtiss, L. K., and Witzum, J. L. [1987] J. Clin. Invest. 79, 1831–1841; Young, S. G., Bertics, S. J., Curtiss, L. K., Dubois, B. W., and Witzum, J. L. [1987] J. Clin. Invest. 79, 1842–1851). Apolipoprotein B37 contains only the amino-terminal portion of apo-B100. In affected individuals most of the apo-B37 is contained in the high density lipoprotein (HDL) fraction (d = 1.063–1.21 g/ml), where it is the principal apolipoprotein in a unique lipoprotein (Lp-B37), which contains little, if any, apo-A-I. However, the most abundant lipoprotein in the HDL density fraction is a smaller particle, which contains apo-A-I, but no apo-B.

The Lp-B37 particles were isolated from the HDL of affected individuals by immunoadsorption of apo-B37. Selected affinity antibodies specific for apo-B37 were used to prepare an anti-apo-B37-Sepharose 4B column. Lipoproteins not bound by the column (unbound HDL fraction) contained apo-A-I, but no apo-B. The Lp-B37, which was eluted from the column with 3 M KI, contained apo-B37 and trace amounts of apo-A-I, but no apo-B100. Over a 4-h period, normal human fibroblasts degraded 10-fold more 125I low density lipoprotein (LDL) than 125I-Lp-B37. Also, whereas addition of excess unlabeled LDL markedly reduced degradation of 125I-LDL, it did not significantly reduce the degradation of 125I-Lp-B37. Unlabeled Lp-B37 did not inhibit uptake and degradation of 125I-LDL by fibroblasts. These data suggest that the amino-terminal portion of apo-B100, when expressed on a naturally occurring lipoprotein particle, does not contain a functional apo-B,E(LDL) receptor binding domain.

Apolipoprotein (apo-) B100 plays a central role in human lipoprotein metabolism. It is the predominant protein in human low density lipoproteins (LDL) and is the ligand responsible for the receptor-mediated uptake of LDL by cells (1, 2). Because of the large size of apo-B100, its insolubility, and its propensity to break down into a number of smaller polypeptides, there has been, until recently, little information on its structure and functional domains. However, within the past year, the complete nucleotide sequence of the apo-B cDNA has been determined by four independent groups (3–6). The derived amino acid sequence of apo-B will be invaluable in understanding apo-B structure and function. An important issue will be to localize the apo-E,E(LDL) receptor binding domain(s) of apo-B as precisely as possible.

Several clues about the probable nature of the apo-B receptor binding domain already exist. The structure and functional domains of apo-E, the other ligand for the apo-B,E(LDL) receptor, have been extensively studied (2, 7, 8). The receptor binding domain of apo-E is centered near amino acid residues 140–150 and is rich in lysine and arginine residues. Chemical modification of the lysine and arginine residues of apo-E abolishes its ability to bind to the apo-B,E(LDL) receptor (9, 10).

Chemical modification of the lysine and arginine residues of apo-B also disrupts LDL binding, suggesting that these positively charged amino acids may play a critical role in the receptor binding domain of apo-B100 (10, 11). Two basic amino acid sequences in the carboxy-terminal region of apo-B (residues 3147–3157 and 3359–3367) have been proposed as candidates for the receptor binding domain of apo-B (3, 12). The second of these sequences has partial homology to the receptor binding domain sequence of apo-E.

Studies with apo-B-specific monoclonal antibodies support the idea that these two carboxy-terminal amino acid sequences, or at least sequences close by, may actually be
important for receptor binding. Some apo-B-specific antibodies are capable of blocking the binding of LDL to the apo-B,E(LDL) receptor, whereas other antibodies specific for apo-B do not block binding (13-16). The epitopes for several of the receptor-blocking antibodies have been localized to sequences flanking the two basic amino acid regions (3). For example, antibody MB47, which was developed and characterized in our laboratory (15), has been shown to bind to apo-B within residues 3350-3505 (3). The epitopes of two other receptor-blocking antibodies, 4G3 and 3F5 (13, 14), bind within residues 3037-3132 (3). Recently, it has been shown that the two basic regions may be brought closer to one another as a result of a disulfide bridge between residues 3167 and 3297 (3).

In spite of strong immunoechemical evidence localizing the receptor binding domain to the carboxyl-terminal region of apo-B100, uncertainty about the nature and location of the receptor binding domain has persisted. Proctor et al. (17) have pointed out the existence of sequences rich in basic amino acids in the amino-terminal region of apo-B100; the functional significance of these sequences, if any, has not been determined. Recently, Hospatrankar et al. (18) proposed that 12 widely dispersed regions of apo-B100, including some amino-terminal sequences, may be involved in receptor binding.

Localization of the receptor binding domain of apo-E was greatly aided by a detailed multidisciplinary examination of clinically important mutations in apo-E (2, 7, 8). Similarly, examination of mutations in apo-B will likely be rewarding in localizing the receptor binding domain of apo-B100. In this study, the binding characteristics of a lipoprotein containing a mutant apo-B were examined; the results yield insights into the location of the receptor binding domain of apo-B100.

In 1979, Steinberg et al. (19) described a unique kindred with familial hypobetalipoproteinemia. In a further evaluation of this kindred, we found that the lipoproteins of nine family members with hypobetalipoproteinemia had an abnormal species of apo-B, apo-B37 (20, 21). Apolipoprotein B37 contains only the amino-terminal portion of apo-B100 (20). In affected individuals, apo-B37 is found in the high density lipoprotein (HDL) density fraction in a unique lipoprotein, Lp-B37. These particles contain apo-B37, but no apo-B100, and little, if any, apo-A-I. They constitute only a small fraction of lipoproteins found in the HDL density range.

In this study, we successfully purified Lp-B37 particles from the HDL density fraction of several affected family members. The ability of 125I-Lp-B37 particles to be degraded by cultured human fibroblasts and the ability of unlabeled Lp-B37 particles to compete with intact 125I-LDL for cellular uptake and degradation was tested. No significant uptake of Lp-B37 particles was observed. Thus, the amino-terminal portion of apo-B100, at least as expressed in Lp-B37, a naturally occurring lipoprotein particle, does not appear to contain a physiologically important receptor binding domain.

MATERIALS AND METHODS

Human Subjects

The H. J. B. kindred was originally described by Steinberg et al. (19). Recr co-workers (20, 21) further examined the kindred and found evidence for two abnormal apo-B alleles, one encoding for a truncated apo-B species, apo-B37, and a second associated with low plasma concentrations of apo-B100. A family tree, in which each family member is identified by a number and characterized according to apo-B genotype, has been published (21). Fresh plasma samples were obtained from selected family members whose lipoproteins contained apo-B37: subjects 1 (H. J. B.), 3, 13, and 14, as previously identified (21). Blood was collected into tubes containing EDTA (1.5 mg/ml of blood), and plasma was immediately isolated by centrifugation at 4°C. Multiple proteolytic inhibitors were then added to the plasma, as previously described (20). Control plasma was obtained from normal laboratory personnel.

Antibodies

Four murine monoclonal antibodies specific for apo-B were used in this study: MB47, MB19, MB3, and MB11. Antibody MB47 (15) binds to apo-B100, but not to apo-B48 or apo-B37 (20). Antibodies MB19, MB3, and MB11 (15, 24-27) bind to apo-B100, apo-B48, and apo-B37 (20). We also used a murine monoclonal antibody, A-1-10 (28), specific for apo-A-I, and a murine monoclonal antibody, E5, specific for apo-E.

Polyclonal antisera to apo-B37 were developed in rabbits (20). The apo-B37 used for immunization was purified from the HDL of subject 1 by preparative sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (29). On analytical SDS-polyacrylamide gel electrophoresis, the immunogen contained apo-B37, but no apo-E or apo-A-I. By Western blot analysis, the resultant antisera bound to apo-B37, apo-B100, and apo-B48, but not to apo-E or apo-A-I (20).

Production and Use of the Anti-apolipoprotein B37 Immunoblotting Column

The techniques for immunoblotting of Lp-B37 from HDL are similar to those previously reported by McVicar et al. (30) for immunoblotting of apo-A-I from plasma. Forty milligrams of LDL protein were covalently bound to 6 g of CNBr-activated Sepharose 4B (Pharmacia Biotechnology, Inc.) according to manufacturer's instructions, and a 6 × 2-cm LDL-Sepharose column was prepared. After the column was washed with PBS, 10 ml of anti-apo-B37 rabbit serum was circulated slowly (0.1 ml/min) over the column for 14 h at 4°C; the column was then washed with PBS until no more protein could be removed, as determined by a UV monitor at 280 nm. Then 1 M acetic acid, pH 2.8, was passed over the column at 1.0 ml/min, and elution of antibody was monitored at 280 nm. The eluted antibody was concentrated and neutralized to pH 7.35 with 0.2 M sodium phosphate, dialyzed against PBS, and concentrated on an Amicon Centricon membrane (type CF25; Amicon Corp., Danvers, MA). The average yield of antibody per 10 ml of antisem was 1.5 mg.

Six milligrams of purified antibody was bound to 2 g of CNBr-activated Sepharose 4B according to manufacturer's instructions, and a 3 × 2-cm anti-apo-B37 column was prepared. The column was washed extensively with PBS.

To isolate Lp-B37 particles, 10 mg of LDL from an affected family member was circulated over the column for 14 h at 4°C. Then PBS was pumped over the column at 1.0 ml/min, and lipoproteins not bound to the column (unbound HDL fraction) were collected. Phosphate-buffered saline was pumped over the column for 30-60 min after protein could no longer be detected by the UV monitor. The Lp-B37 particles then were eluted from the column with 3 M KI. The eluate was immediately transferred to a dialysis bag and dialyzed against multiple changes of PBS for 48 h.

Characterization of the Lp-B37 Preparation

Chemical Composition—The protein concentration of Lp-B37 was determined by a modification of the Lowry technique (23). Phospholipid concentration was determined using a micromodification of the Bartlett procedure (31). Total cholesterol concentration and triglyceride concentration were determined enzymatically.

Apolipoprotein Content—The apolipoprotein content of Lp-B37 was assessed by SDS-polyacrylamide slab gel stained with Coomassie Brilliant Blue (24). Identity of individual apolipoproteins was confirmed by Western blot analysis with monospecific antibodies as previously described (24, 27).

The apo-B content of Lp-B37 was assessed by solid phase competitive radioimmunoassay (RIA) using apo-B-specific monoclonal antibodies or the polyclonal antibody to apo-B37. This assay was
performed exactly as previously described (15, 26). Briefly, flexible 96-well plates were coated with PBS containing 10 μg of control LDL/ml. The remaining binding sites were blocked by coating with PBS containing 4% bovine serum albumin. Control LDL was utilized for the standard curve, which was included on each plate. The control LDL and other lipoprotein preparations (including Lp-B37) were diluted in human serum albumin, 0.05% Tween 20, and 0.05% sodium azide, and 0.05% Tween 20 to concentrations ranging from 0.23 to 123 μg/ml. The control LDL and other lipoprotein competitors (25 μl) were added to the LDL-coated wells, followed by 25 μl of a fixed and limiting amount of antibody. After an 18-h incubation at 4°C, the wells were washed, and the amount of the first antibody bound to the immobilized LDL was quantitated by the liquid-phase 

**Lipoprotein Sizing**—The size distribution of LP-B37 particles was determined by gradient polycrylamide gel electrophoresis under non-denaturing conditions using commercially available gels (PAA 4/30 gels, Pharmacia Biotechnology, Inc.), as previously described (20, 32). Gels were stained with silver or were electrophoretically blotted onto nitrocellulose sheets for Western blot analysis (20).

*Studies with Cultured Fibroblasts*

The uptake and degradation of 125I-labeled lipoproteins by normal human foreskin fibroblast monolayers were assessed essentially as previously described (15, 33-35).

Normal human foreskin fibroblasts were grown in 22- or 35-mm wells in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum for 3-4 days. The cells were then washed, and the medium was changed to DMEM containing 10 mg of lipoprotein-depleted serum (LPDS)/ml. After 48 h in DMEM-LPDS, when the cells had reached 85% confluency, the uptake and degradation experiments were performed.

Low density lipoproteins (d = 1.019-1.063) isolated from pooled normal plasma and Lp-B37 isolated from the HDL density fraction of subject 1 were iodinated using the IODO-GEN technique (36). The iodinated lipoproteins were then dialyzed for 48 h against multiple Tris-buffered saline (TBS) and a fainter apo-A-I band were consistently observed. No apo-A-I was detectable in the Lp-B37 preparation (Fig. 1, panel C). Western blots using an apo-A-I-specific antibody demonstrated a dense apo-B37 band. The apo-B37-specific antibodies were first selected for their ability to bind to and be readily eluted from an LDL-Sepharose column. The apo-apo-B37 antibodies were eluted from the LDL column with 1 μM acetic acid, pH 2.8. These antibodies were then used to prepare an anti-apo-B37-Sepharose column. The whole HDL fraction from one of the affected subjects was then circulated over the column. High density lipoprotein particles not bound by the column (the unbound HDL fraction) contained no apo-B37 (Fig. 1), indicating that the column was effective in removing the Lp-B37 particles from the whole HDL preparation. Our initial experiments indicated that 1 μM acetic acid, pH 2.8, was ineffective in eluting all Lp-B37 particles from the anti-apo-B37 column. When 3 μM KI was used to elute the column, the yield of Lp-B37 particles was far greater. After dialysis and concentration of the sample on an Amicon filter, we obtained yields of 300 μg of Lp-B37 protein for each 10 mg of HDL loaded onto the column.

*Characterization of Purified Lp-B37*—The Lp-B37 preparations utilized in the cell culture experiments described below were extensively characterized. We determined the protein, cholesterol, and phospholipid concentrations of normal HDL, HDL of subject 1, and the unbound HDL fraction and Lp-B37 preparations isolated from the HDL fraction of subject 1. The phospholipid/protein and cholesterol/protein ratios of these lipoproteins are summarized in Table I. Triglycerides accounted for less than 5% of Lp-B37 mass. The chemical composition of Lp-B37 particles was similar to that of normal HDL.

When the purified Lp-B37 preparations were subjected to SDS-polyacrylamide gel electrophoresis and the gel was stained with Coomassie Brilliant Blue, a dense apo-B37 band and a fainter apo-A-I band were consistently observed. No apo-E was observed (data not shown). Western blots of the SDS-polyacrylamide gels, using specific monoclonal antibodies, were used to identify the apolipoproteins in the Lp-B37 preparations. The results of the Western blots of purified Lp-B37 from subject 1 are shown in Fig. 1. A Western blot using antibody MB19 demonstrated a dense apo-B37 band. A faint apo-B26 band was observed, but no other breakdown products were seen (Fig. 1, panel A). A Western blot using an apo-A-I-specific antibody demonstrated a small amount of apo-A-I in the Lp-B37 preparations (Fig. 1, panel B). No apo-E was detectable in the Lp-B37 preparation (Fig. 1, panel C). Western blots were also performed with the Lp-B37 preparations from subjects 1, 3, and 13, and similar results were obtained. However, a small amount of apo-E was detectable in the Lp-B37 preparations isolated from the HDL of subjects 3 and 13. Consequently, the Lp-B37 preparations from these two subjects were not used in the cell culture experiments described below.

The ability of the purified Lp-B37 preparation to compete...
Characterization of a Lipoprotein Containing Apo-R37

A. MB19

B. A-I-10

C. E5

FIG. 1. Western blots demonstrating apolipoprotein content of Lp-B37 purified from the HDL of subject 14. Thirty micrograms of each lipoprotein was applied to a 3-15% polyacrylamide slab gel containing 0.1% SDS. Following electrophoresis, the separated apolipoproteins were transferred to nitrocellulose membranes, and Western blots were performed (27). Panel A demonstrates the binding of the apo-B-specific monoclonal antibody MB19 to the whole HDL of subject 14 (lane 1), the unbound HDL fraction (lane 2), and the Lp-B37 (lane 3). Panel B demonstrates the binding of the apo-A-I-specific monoclonal antibody A-I-10 to the HDL of subject 14 (lane 1), the unbound HDL fraction (lane 2), and the Lp-B37 (lane 3). The film was overexposed to show the presence of a small amount of apo-A-I in the Lp-B37 preparation. Panel C demonstrates the binding of the apo-E-specific monoclonal antibody E5 to the unbound HDL fraction (lane 1) and Lp-B37 (lane 2) obtained from the HDL of subject 14. Lane 3 of panel C shows the binding of E5 to chylomicrons obtained from the blood of a normal subject.

TABLE I

Chemical composition of Lp-B37

|                  | Phospholipid/protein ratio | Cholesterol/protein ratio |
|------------------|-----------------------------|---------------------------|
| Control HDL      | 0.59                        | 0.50                      |
| H. J. B. HDL     | 0.61                        | 0.32                      |
| Unbound HDL fraction | 0.59                    | 0.36                      |
| Lp-B37           | 0.46                        | 0.42                      |

*Mean of duplicate determinations.

with LDL for binding to apo-B-specific antibodies was assessed in solid-phase RIAs. Fig. 2A illustrates the results obtained with the RIA using antibody MB11. In this assay the lipoprotein competitors were added on the basis of their protein content. Lp-B37 competed with the immobilized LDL for binding to the antibody MB11 (an amino-terminal-specific apo-B monoclonal antibody) slightly better than did native LDL. A likely reason for this finding is that, compared with LDL, there is a higher molar concentration of Lp-B37 particles when they are added to the assay at the same protein concentration. No competition was observed with the unbound HDL fraction, indicating that the anti-apo-B37 column had effectively removed nearly all the apo-B from the HDL. Assessment of the apo-A-I content of the Lp-B37 preparations in a sensitive immunossay showed that apo-A-I constituted, at most, 13% of the Lp-B37 protein. Purified Lp-B37 did not bind to antibody MB47, indicating that the purified Lp-B37 did not contain any apo-B100 (Fig. 2B).

The purified Lp-B37 particles had the same size as Lp-B37 particles contained within HDL, as determined by gradient polyacrylamide gel electrophoresis under nondenaturing conditions (Fig. 3). There was no evidence for aggregation of the Lp-B37 preparation on the nondenaturing gels. On 1% agarose gels, the purified Lp-B37 particles had essentially the same pre-β mobility as did Lp-B37 particles in the HDL density fraction (data not shown).

Electron microscopy of the purified Lp-B37 particles demonstrated that they were larger than the apo-A-I-containing particles in the unbound HDL fraction. Representative elec-

FIG. 2. Ability of Lp-B37 to compete with LDL for binding to apo-B-specific antibodies in a solid phase RIA. Panel A shows an RIA utilizing antibody MB11, and panel B shows an RIA utilizing antibody MB47. The following lipoproteins were tested for their ability to compete with immobilized LDL for binding to four different apo-B-specific antibodies: control LDL (○), control HDL (●), subject 13's whole HDL fraction (■), Lp-B37 from subject 13 (■), and unbound HDL fraction from subject 13 (▲). Each lipoprotein competitor was added to the assay on the basis of its protein content. B and B0 specific counts bound in the presence or absence of competitor, respectively. Similar results were obtained with HDL, Lp-B37, and unbound HDL fraction preparations from subjects 3 and 14. Also, similar results were obtained in other assays using other apo-B-specific antibodies (MB3, MB19) that bind to the amino-terminal portion of apo-B100.
shown was stained with silver. Polyacrylamide gels.

apo-3.

30,

ditions

prepared under nondenaturing conditions demonstrated that the Lp-37 particles, 126 and 14. Western blots of identical gels electrophoresed under nondenaturing conditions demonstrated that the Lp-37 preparations contained apo-B and only trace amounts of apo-A-I. These data strongly support the idea that apo-B does not contain an LDL receptor binding domain.

Further evidence that Lp-B37 did not bind to the LDL receptor of fibroblasts was obtained with an Lp-B37 preparation that was radioiodinated and tested directly for uptake and degradation by cultured fibroblasts. Whereas degradation of LDL showed the usual high-affinity receptor-mediated curve, degradation of 125I-Lp-B37 was very low and linear, typical of nonspecific degradation.

ment, we tested the ability of unlabeled control HDL and unlabeled HDL from subject 1 to compete with 125I-LDL for degradation by fibroblasts (Fig. 5). Even at a very high concentration of competitor, HDL from subject 1 competed no better than control HDL. Our RIA results (Fig. 2) suggested that Lp-B37 constitutes ~5% of the HDL mass. Thus, at the 1000 μg/ml point, there was ~50 pg of Lp-B37/ml. However, whole HDL from an affected subject, even at 1000 μg/ml, failed to compete with 125I-LDL any better than control HDL.

In contrast, very low concentrations of unlabeled LDL competed quite well with 125I-LDL for degradation by fibroblasts. Next, purified Lp-B37 preparations were tested for their ability to compete with 125I-LDL for uptake and degradation by cultured fibroblasts. Three different Lp-B37 preparations were tested in three different experiments (Fig. 6). Fig. 6 includes the results obtained by analyzing the 125I-labeled degradation products in the media (striped bars) as well as the 125I-LDL cell-associated radioactivity (white bars). Both the degradation products and cell-associated radioactivity yielded the same results. Even at high competitor concentrations, Lp-B37 particles failed to compete with 125I-LDL for uptake and degradation. In fact, they did not compete any better than whole HDL or the unbound HDL fraction, which contained very little, if any, apo-B. These data strongly support the idea that apo-B does not contain an LDL receptor binding domain.

FIG. 5. Ability of normal HDL and HDL from subject 1 to compete with 125I-LDL for degradation by cultured fibroblasts. Normal human fibroblast monolayers were grown in 35-mm wells in DMEM containing 10% fetal calf serum. Fibroblast apo-B,E(LDL) receptor activity was stimulated by a 24-h preincubation with DMEM containing 10 mg of LPDS/ml of medium. Normal human LDL was iodinated with the IODO-GEN (Pierce Chemical Co.) technique (36) and then extensively dialyzed against PBS. The specific activity of the 125I-LDL was 187 cpm/ng of LDL protein. The DMEM-LPDS medium was removed from the cells, and new DMEM containing 2.0 μg of 125I-LDL/ml, 10 mg of LPDS/ml, and increasing concentrations of lipoprotein competitor were then added to the cells. Exactly 1 ml of medium was added to each well. In this experiment, competitors included normal HDL (A), LDL from subject 1 (B), and normal LDL (C). All determinations were made in duplicate. After a 5-h incubation, 125I-LDL degradation products in each medium were counted after precipitation of 125I-LDL and free 125I with trichloroacetic acid and silver nitrate, respectively (34). Control wells containing no competitor degraded 0.6 μg of 125I-LDL/mg of cell protein during the 5-h incubation. Shown here is the percent of control degradation (y axis) versus lipoprotein competitor (x axis) added to the medium. The average amount of cell protein/well was 150 μg.

A.

B.

FIG. 4. Electron microscopy of unbound HDL fraction particles and Lp-B37 particles. Panel A shows particles in the unbound HDL fraction from subject 1; panel B shows Lp-B37 particles from subject 1. Both electron micrographs are shown here at 50,000 × magnification. The unbound HDL fraction particles were 91 ± 10 Å in diameter; the Lp-B37 particles, 126 ± 11 Å. Similar results were obtained with the Lp-B37 particles and unbound HDL fraction particles from subject 3.
also competes with familial hypobetalipoproteinemia (19). Further evaluation of the Lp-B37 particles to 3McKI, whereas the control LDL had not been subjected to 3M KI. However, it is unlikely that the brief exposure of the Lp-B37 particles to 3M KI destroyed a physiologically important binding domain on the Lp-B37 particles because incubation of control LDL with 3M KI for 2 h did not significantly affect its ability to be taken up and degraded by fibroblasts (data not shown).

**DISCUSSION**

In 1979, Steinberg and colleagues reported a unique kindred with familial hypobetalipoproteinemia (19). Further evaluation of that kindred provided evidence for two abnormal apo-B alleles associated with hypobetalipoproteinemia (20, 21). One allele was associated with extremely low plasma concentrations of apo-B100. The other abnormal allele yielded an abnormal species of apo-B, apo-B37 (M, 205,000). Previously, we demonstrated that apo-B37 contains only the amino-terminal portion of apo-B100 (i.e., apo-B37 is a truncated version of apo-B100). Based on its apparent molecular weight, we estimate that apo-B37 must end near amino acid residue 1700. Recently, we have shown that polyclonal antibodies to a synthetic apo-B peptide (residues 2008–2024) bind to apo-B48 and apo-B100, but not to apo-B37.

In affected individuals, apo-B37 is the principal lipoprotein particle, Lp-B37, which is present in the HDL fraction. In the present study, the Lp-B37 particles were purified from HDL by using an anti-apo-B37 immunoaffinity column. The approach and techniques were very similar to those utilized by McVicar et al. (30) for absorption of apo-A-1-containing particles from plasma. Our column was effective in removing all apo-B37-containing particles from HDL; no significant amounts of apo-B were detectable in the unbound HDL fraction by RIA (Fig. 2). The column was used repeatedly without apparent loss of ability to bind Lp-B37. The mobility of immunopurified Lp-B37 particles in non-

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3 Innerarity, T. L., Young, S. G., Poksay, K. S., Mahley, R. W., Smith, R. S., Milne, R. W., Marcel, Y. L., and Weisgraber, K. H. (1987) *J. Clin. Invest.* in press.

4 S. Young and B. Dubois, unpublished observations.
FIG. 7. Ability of $^{125}$I-LDL and $^{125}$I-Lp-B37 to be degraded by cultured human fibroblasts. Fibroblasts were prepared in 22-mm wells as described in the legend to Fig. 5. Radioiodination of lipoproteins and measurement of $^{125}$I-lipoprotein degradation products were performed after a 4-h incubation as described in the legend to Fig. 5. Fibroblasts were incubated with DMEM containing 10 μg of LPDS/ml and increasing concentrations of control $^{125}$I-LDL (●) or $^{125}$I-Lp-B37 (●) purified from the HDL of subject 1. When 500 μg of unlabelled LDL/ml was added to the medium at each concentration of $^{125}$I-LDL, there was a marked decrease in degradation of $^{125}$I-LDL (●). When 500 μg of unlabelled LDL/ml was added to the medium at each concentration of $^{125}$I-Lp-B37, there was essentially no change in the degradation of $^{125}$I-Lp-B37 (●). All determinations were performed in duplicate. The amount of fibroblast protein/well was 80–100 μg. Results with cell-associated $^{125}$I also indicated little specific uptake of $^{125}$I-Lp-B37, compared with $^{125}$I-LDL (see "Results").

naturing gradient polyacrylamide gels and agarose gels was identical to that of Lp-B37 particles in the whole HDL fraction. There was no evidence that the purification scheme caused aggregation of the particles. Apolipoprotein B37 was not degraded during the purification process (Fig. 1).

Each of the experiments with cultured fibroblasts suggested that Lp-B37 had little ability to be taken up by the apo-B,E(LDL) receptor. Lp-B37 competed with $^{125}$I-LDL for uptake by fibroblasts no better than the unbound HDL fraction, which contained no apo-B. The very low levels of competition observed with the unbound HDL fraction and with Lp-B37 (Fig. 6) may have been nonspecific or may have been due to the extremely low concentrations of apo-E in the unlabeled lipoprotein competitors.

Recently, Corsini et al. (38) prepared recombinant lipoprotein particles containing large thrombolytic fragments of apo-B100. Thrombin cleaves LDL-apo-B100 into three fragments: T4 (residues 1–1297), T3 (residues 1298–3249), and T2 (residues 3250–4536) (3, 12, 20). Denatured thrombolytic fragments were purified from preparative SDS-polyacrylamide gels and used to form recombinant particles. Recombinant particles containing each of the three thrombin fragments bound specifically to the apo-B,E(LDL) receptor. Corsini et al. (38) recommended caution in interpreting these studies as demonstrating physiologically significant binding domains in multiple regions of apo-B100. They pointed out that the series of steps involved in making the recombinant particles (i.e. SDS denaturation of the thrombolytic fragments) could potentially unmask sequences capable of binding to the apo-B,E(LDL) receptor that were not normally exposed on native LDL particles. In our studies, we examined the binding of a naturally occurring lipoprotein particle containing the amino-terminal portion of apo-B100 and found no specific binding to the apo-B,E(LDL) receptor.

While providing no direct evidence localizing the apo-B,E(LDL) receptor binding domain to the carboxyl-terminal region of apo-B100, our data are quite consistent with immunoechemical evidence suggesting that the receptor binding domain of apo-B100 is contained in the carboxyl-terminal region of apo-B100 near the T3-T2 cleavage point (3, 12, 20). Our data make the possibility of a receptor binding domain in the amino-terminal portion of apo-B100 seem unlikely.

Our previous studies with monoclonal antibodies have suggested that apo-B48 contains only the amino-terminal portion of apo-B100 (20). Studies with polyclonal antibodies to synthetic apo-B peptides have confirmed this finding. Hui et al. (39) reported in vivo metabolic studies and in vitro cell culture experiments demonstrating that apo-B48 has no role in the receptor-mediated uptake of triglyceride-rich lipoproteins, suggesting that apo-B48 does not contain a receptor binding domain. The disadvantage of using the apo-B48-containing particles is that they are large triglyceride-rich lipoproteins with significant amounts of other apolipoproteins, including apo-E. In contrast, Lp-B37 particles are cholesterol-rich, triglyceride-poor particles with very low levels of apo-E. Both the Hui et al. study and this study are consistent in suggesting that there is no physiologically important LDL receptor binding domain in the amino-terminal portion of apo-B100.

We believe that studies of other apo-B mutations will yield new insights about the location and the structure of the receptor binding domain of apo-B100. Recently, Vega and Grundy (40) reported the existence of hypercholesterolemic patients whose LDL are cleared from plasma more slowly than LDL isolated from a normal subject. It seems likely that some of these patients may have a defect in the receptor binding domain of their apo-B100. Detailed study of such patients should yield additional insights into the receptor binding domain of apo-B100.

Acknowledgments—We thank Daniel Steinberg for his advice and support and for his encouragement to study the H. J. B. Kindred. We thank the H. J. B. kindred for their interest and participation. We acknowledge the help of Lorraine Ware, a recipient of a summer student fellowship award from the American Heart Association, California Affiliate. We thank Richard Elam and Elizabeth Miller for technical support, Dr. Richard Smith for performing the immunoassays of apo-A-I, and Debra Coller for manuscript preparation.

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