Apolipoprotein L, a New Human High Density Lipoprotein
Apolipoprotein Expressed by the Pancreas

IDENTIFICATION, CLONING, CHARACTERIZATION, AND PLASMA DISTRIBUTION OF APOLIPOPROTEIN L

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In this study, we have identified and characterized a new protein present in human high density lipoprotein that we have designated apolipoprotein L. Using a combination of liquid-phase isoelectrophoresis and high resolution two-dimensional gel electrophoresis, apolipoprotein L was identified and partially sequenced from immunosolated high density lipoprotein (Lp(A-I)). Expression was only detected in the pancreas. The cDNA sequence encoding the full-length protein was cloned using reverse transcription-polymerase chain reaction. The deduced amino acid sequence contains 383 residues, including a typical signal peptide of 12 amino acids. No significant homology was found with known sequences. The plasma protein is a single chain polypeptide with an apparent molecular mass of 42 kDa. Antibodies raised against this protein detected a truncated form with a molecular mass of 39 kDa. Both forms were predominantly associated with immunoaffinity-isolated apoA-I-containing lipoproteins and detected mainly in the density range 1.123 < d < 1.21 g/ml. Free apoL was not detected in plasma. Anti-apoL immunoaffinity chromatography was used to purify apoL-containing lipoproteins (Lp(L)) directly from plasma. Nondenaturing gel electrophoresis of Lp(L) showed two major molecular species with apparent diameters of 12.2–17 and 10.4–12.2 nm. Moreover, Lp(L) exhibited both pre-β and α electromobility. Apolipoproteins A-I, A-II, A-IV, and C-III were also detected in the apoL-containing lipoprotein particles.

Epidemiological studies have demonstrated a strong inverse correlation between the levels of plasma high density lipoproteins (HDL) and risk of premature coronary heart disease (1, 2). However, the mechanisms by which HDL protect against atherosclerosis need further exploration. One proposed protective role of HDL involves reverse cholesterol transport (3–5), a process in which HDL acquire cholesterol from peripheral cells and facilitate its esterification and delivery to the liver. In this process, small, relatively lipid-poor HDL particles, termed pre-β-HDL, have been postulated to be the first acceptors of cholesterol from the cells (4, 6, 7). An additional mechanism may involve the ability of HDL to impede the oxidation of other plasma lipoproteins (8–10).

A major difficulty in understanding HDL metabolism is the molecular heterogeneity of HDL (11, 12). Until recently, ultracentrifugation was the most practical way to purify HDL. This methodology has been the basis for the vast majority of the studies in this field. However, it is now well documented that ultracentrifugation causes protein dissociation and can modify structures of HDL particles (13–15). An alternative purification strategy that conserves lipoprotein integrity is immunoaffinity chromatography, which isolates lipoproteins on the basis of their protein content (15–17). The development of the strategy of selected affinity immunosorption is particularly suited to investigation of the protein constituents of lipoprotein complexes because it permits isolation of the lipoproteins under minimally perturbing conditions (17). For example, functional components such as lecithin:cholesterol acyltransferase and cholesterol ester transfer protein are present in higher concentrations in immunopurified lipoproteins, whereas they are depleted or absent in ultracentrifugally purified lipoproteins (12, 15, 18). These observations demonstrate the importance of immunofinity chromatography in identifying novel HDL-associated proteins of potential physiological significance.

In this study, we employed selected affinity immunosorption and two-dimensional gel electrophoresis to identify a new protein we have designated apolipoprotein L (apoL) that is associated with plasma lipoproteins, predominantly with apoA-I-containing lipoproteins (Lp(A-I)). We report here the isolation and plasma lipoprotein distribution of apoL and the cloning and characterization of the cDNA encoding apoL.

EXPERIMENTAL PROCEDURES

Isolation of Lipoproteins—Blood was drawn from fasting normolipidemic subjects (female and male) and immediately cooled to 4 °C in the presence of preservatives and protease inhibitors (0.04% EDTA, 0.05% NaN₃, 1 μg/ml gentamycin, 0.3 mg/ml benzamidine, 1 mM phenylmethylsulfonyl fluoride, 0.13% e-amino-n-caproic acid, and 10 μg/ml aprotinin, final concentrations) (12). Plasma was separated by centrifugation at 1000 × g for 45 min at 4 °C.

The apoA-I-containing lipoproteins (Lp(A-I)) were isolated by selected affinity immunosorption (17). Plasma was applied to a selected affinity anti-apoA-I column. The unbound fraction was eluted with Tris-buffered saline (5 mM Tris (pH 7.4), 150 mM NaCl, 0.04% EDTA, and 0.05% NaN₃). The Lp(A-I) fraction was eluted with 0.2 M acetic acid (pH 3) and 0.15 M NaCl. The eluate was immediately neutralized to pH 7.4 with 2 M Tris (Tris base), and preservatives were added as described above. Finally, Lp(A-I) were passed through protein A-Sepharose and anti-albumin columns to remove traces of albumin and immunoglobulins.

The apoL-containing lipoproteins (Lp(L)) were isolated similarly.
First, 200 µg of apoL was purified by electroelution from two-dimensional gels. The purified protein was used to raise rabbit antisera. Antibodies were adsorbed to protein A-Sepharose, and the IgG fraction was eluted with 0.2 M acetic acid and neutralized with 2 M Tris. The IgG fraction was cross-linked to CNBr-activated Sepharose (Pharmacia, Upjohn) to construct an anti-apoL protein affinity column. To confirm the identity of apoL by protein blotting, VLDL (d < 1.006 g/ml), LDL (1.006 < d < 1.019 g/ml), HDL (1.019 < d < 1.063 g/ml), HDL1 (1.063 < d < 1.123 g/ml), and HDL2 (1.123 < d < 1.21 g/ml) fractions were isolated from plasma by sequential ultracentrifugation in a Beckman 40.3 rotor (10 °C, 36,000 rpm, 19 h) (19). Solvent densities were adjusted with anhydrous KBr and verified by pycnometry. After isolation, lipoproteins were dialyzed against Tris-buffered saline.

Identification of ApoL—ApoL was purified by a combination of preparative liquid-phase isoelectric focusing (ROTOFOR, Bio-Rad) and high resolution two-dimensional gel electrophoresis (20). 200 mg of Lp(A-I) was fractionated with the ROTOFOR into 20 fractions over a pH range of 3.5–10.0. Fractions of interest were then subjected to two-dimensional gel electrophoresis. After electrophoresis, the proteins were electrotransferred to a polyvinylidene difluoride membrane (Bio-Rad). Proteins were stained with Coomassie Blue, and individual spots were subjected to N-terminal sequence analysis (Model 473 A, Applied Biosystems, Inc., Foster City, CA) (21). The amino-terminal sequences were compared with the SWISS-PROT and GenBank™ data bases (22).

Northern Hybridization—A multiple-tissue Northern blot (CLONTECH, Palo Alto, CA) was probed, strictly adhering to the recommended protocol. Each lane contained 2 µg of highly purified poly(A) RNA from various human tissues. The Northern blot was probed with a synthetic guessmer (5'-AAGGGGTTTCTGGGGA/GATGCTCGTG- (GA/TCTGTGCGCAGGGACATTTCTGCTGACAC/TG/GGGCGCCA- GCCCTCTCCG-3') that corresponds to the first 25 residues of the circulating form of apoL.

cDNA Cloning—We used a reverse transcription-polymerase chain reaction (PCR)-based cDNA cloning strategy (23) to isolate a cDNA encoding apoL. RNA was prepared from human pancreas using a total RNA isolation kit (CLONTECH). mRNA was purified using an mRNA purification kit (Pharmacia Biotech Inc.). Single-stranded cDNA was synthesized using 1 µg of human pancreas mRNA and 500 ng of oligo(dT) primer 5'-GY(G/A)TCGCCTGT- (AV/C/T/A/G/C/T-3') with 200 units of SuperScript II RNase H- (Life Technologies, Inc.) in a volume of 20 µl. The reaction was then diluted to 200 µl for PCR.

10 µl of single-stranded cDNA was used for amplification of apoL cDNA. The first round of PCR contained 100 ng each of oligo(dT) and primer 321 (5'-CACCTTTCTCCCTGGGTGAGAGTGAG-3') in a final volume of 50 µl. The PCR conditions were 40 cycles of denaturation for 30 s at 95 °C, annealing for 50 µl at 55 °C, extension for 1 min at 72 °C, and a final extension for 7 min. An aliquot (0.5 µl) of this product was used in a second round of PCR using 100 ng each of oligo(dT) and primer 329 (5'-GAGGAGCCTGGAGGAGGGTTGCAAC-3') under the same reaction conditions. Oligonucleotides 321, 329, and 331 were designed using the expressed tag DNA sequence recently cloned (24).

A 1.3 kilobase pair, in accordance with the apoL mRNA size on a Northern blot, was extracted from an agarose gel. A third round of PCR was carried out using the same primers as the second. The final PCR product was directly cloned using the pCR-Script™ SK (+) cloning kit (Stratagene, La Jolla, CA). 30 clones were found to have the correct insert. Both strands were sequenced by chain termination using the Thermo Sequenase cycle sequencing kit (Amersham Life Science, Inc.).

Gel Electrophoresis Analysis—One-dimensional SDS-polyacrylamide slab gel electrophoresis (25) was performed employing 12 or 5–25% gradient polyacrylamide gels and run in a Bio-Rad minigel system. Samples were boiled in buffer (2% SDS, 67 mM Tris (pH 6.8), 20% glycerol, and 2% mercaptoethanol) for 1 min before loading. Molecular mass markers (Amersham Corp. or Bio-Rad) were used to calibrate the gels. Gels were stained either with silver nitrate (26) or with Coomassie Brilliant Blue R-250 (Sigma) (27).

Two-dimensional gel electrophoresis (20) was performed with 8.5 × urea in the isoelectric focusing gel. The sample preparation buffer consisted of 9.5 × urea, 0.31% SDS, 2% Nonidet P-40, 100 mM dithiothreitol, and 2% ammonium hydroxide (pH 3–10) (Pharmacia). The apparatus used was the IsoDalt system (Hoefer Scientific Instruments, San Francisco).

Gels were stained with nondenaturing membranes (0.2 µm; Bio-Rad) (28). The membranes were soaked in 10 mM Tris (pH 7.4) and 0.5 mM NaCl with 5% nonfat dry milk and then incubated for 16 h with antiserum. The blots were washed extensively in Tris buffer and incubated for 1 h with horseradish peroxidase-conjugated secondary antibodies. After four washes, proteins were detected by 4-chloro-1-naphthol.

Pre-β- and α-HDL were prepared by starch block electrophoresis of immunopurified Lp(A-I) (29). Briefly, purified potato starch was hydrated with 50 mM barbitol (pH 8.6) and poured into a Plexiglas form. After removal of excess buffer, the starch formed a rigid block. The Lp(A-I) sample (10 mg) was loaded onto the cathodic end, and 200 V was applied across the block. After the lipoprotein had migrated 20 cm as judged by a dye marker (bromphenol blue), the block was fractionated into 1-cm segments, and the apoA-I content of each was determined by immunonephelometry (30). Pre-β- and α-HDL were recovered from the appropriate fractions, and purity was verified by immunoelectrophoresis on 1% agarose with anti-apoA-I antibodies.

Quantitative Assay of ApoL—To quantify apolipoprotein L in plasma, we developed a competitive enzyme-linked immunosorbent assay using IgG purified from rabbit anti-apoL antiserum with purified apoL as a standard. A series of dilutions of plasma were incubated for 16 h at 4 °C with a constant amount of antibody diluted 4000 times with phosphate-buffered saline. The samples were then added to a 96-well plate coated with immunopurified apoA-I-containing lipoprotein (500 ng/well) to quantify the unoccupied antibody. After a 1-h incubation at 23 °C, the plate was washed with phosphate-buffered saline. Horseradish peroxidase-labeled anti-rabbit IgG was added. After 45 min, the plate was washed, and the substrate 3,3',5,5'-tetramethylbenzidine was added. Plates were read at 450 nm using a computer-linked plate reader (Vmax, Molecular Devices, Menlo Park, CA).

RESULTS

Identification of Apolipoprotein L—Through the use of our minimally perturbing method of anti-apoA-I selected affinity immunosorption (17), we have identified subspecies of HDL that contain a new protein that we have designated apolipoprotein L. ApoL was identified from 200 mg of immunosolated Lp(A-I) prepared from normal human plasma. Lp(A-I) were depleted of albumin by anti-albumin immunosorption. Because apoA-I is the predominant protein in the Lp(A-I) fraction, our initial purification step involved preparative liquid-phase isoelectric focusing. This was followed by high resolution two-dimensional gel electrophoresis. Fig. 1A shows a typical fractionation obtained after preparative isoelectric focusing.

FIG. 1. Apolipoprotein L purification from Lp(A-I). Lp(A-I) protein (200 mg) was resolved by liquid-phase isoelectric focusing into 20 fractions representing a pH range of 3.5–10.0. 5 µl of each fraction was then submitted to SDS-PAGE and silver-stained (A). Fractions 9 and 10 were pooled and subjected to two-dimensional gel electrophoresis (isoelectric focusing (IEF)/SDS-PAGE). After electrophoretic transfer to polyvinylidene difluoride membrane, proteins were visualized by Coomassie Blue R-250 staining (B). Individual protein spots were then submitted to microsequencing for identification (arrows show the localization of apoL and apoA-I). ApoL was resolved into two components (indicated by arrowheads).
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Lp(A-I) protein were separated by a combination of liquid-phase isoelectric focusing (Bio-Rad) and two-dimensional gel electrophoresis. After transfer to a polyvinylidene difluoride membrane and staining with Coomassie Blue R-250, N-terminal sequencing was performed on each spot. 15 different samples confirmed these sequences. Using antibodies against the 42-kDa form of apoL, we detected a second form of apoL with an approximate molecular mass of 39 kDa. Clone C22–280 represents the amino acid sequence deduced from the matching DNA sequence found in the SWISS-PROT and GenBank™ data bases.

Table I

N-terminal amino acid sequence of apolipoprotein L and comparison with clone C22–280

| Residues | 12-11-10-9-8-7-6-5-4-3-2-1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 |
|----------|-----------------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 42-kDa protein | E E A G A R V Q Q N V P S G T D T G D P Q S K P L G D | D W A A G T M D P E |
| 39-kDa protein | M S A L F L G V R V R A E E A G A R V Q Q N V P S G T D T G D P Q S K P L G D | |
| C22–280 clone | |

ApoA-I, the most predominant band in fraction 6, was greatly depleted in succeeding fractions. Fractions enriched in apoL (fractions 9–10) were then submitted to analytical two-dimensional gel electrophoresis, and the resolved proteins were electrotransferred to polyvinylidene difluoride membranes (Fig. 1B). Each Coomassie Blue-stained spot was submitted to N-terminal microsequencing, and their sequences were compared with known proteins. The first 27 amino acid residues were identified (Table I). This sequence was confirmed in 15 samples from three donors. By analytical two-dimensional gel electrophoresis, two major proteins (Fig. 1B, indicated by arrows) were determined to have identical N-terminal sequences (Table I). This sequence was compared with those in the SWISS-PROT and GenBank™ data bases. At this time, no sequence matching that of apoL was found. However, during this study, our search for matching sequences revealed the existence of a 143-base pair expressed tag DNA sequence (clone C22–280), recently cloned from human chromosome 22 (24), that matched the apoL sequence. This clone contained 12 amino acid codons upstream of our sequence. Computerized analysis of the sequence using prediction program (31, 32) indicated that these 12 amino acids constitute the signal peptide.

Characterization of ApoL—We prepared apoL antigen by two-dimensional gel electrophoresis, isolating 200 μg by gel electroelution (Fig. 2A). Fig. 2B illustrates the reactivity of rabbit antisera to Lp(A-I). Unexpectedly, the antiserum detected two bands with apparent molecular masses of 42 and 39 kDa. No proteins were detected with preimmune serum (Fig. 2B). The same pattern was obtained under nonreducing and reducing conditions, suggesting the absence of disulfide-bridged subunits. To rule out cross-reaction with other Lp(A-I) proteins, we analyzed the sequence of the 39-kDa protein detected by the antisera. The N terminus of the 39 kDa protein did not correspond, at this time, to any known protein. Later, we found that apol cDNA and part of clone C22–280 (Table I) matched, showing that the 39-kDa protein identified by immunoblotting and amino acid sequence analysis was a truncated form of mature apoL (42 kDa).

Molecular Cloning and Sequence Analysis of ApoL—To clone apol cDNA, we performed a Northern analysis of poly(A)+ RNA from various human tissues (CLONTECH). A single mRNA transcript of ~1.3 kilobase pairs was detected in the pancreas, but not in the heart, brain, placenta, lung, liver, skeletal muscle, kidney, spleen, thymus, prostate, testis, ovary, small intestine, colon, or perileukocytes (Fig. 3). The mark between the ovary and testis lanes in the lower right blot is an artifact.

Using oligonucleotides 331 and 329, an oligo(dT)-primer, and purified pancreas mRNA as a template, we were able to amplify a band of ~1.3 kilobase pairs, as expected according to the Northern analysis (Fig. 3). This was cloned into pCR-Script™ SK (+), and both strands were sequenced. The sequence re-vealated only one possible open reading frame, encoding 383 amino acids with a typical signal peptide of 12 residues and a mature protein of 371 amino acids (Fig. 4). The molecular mass
of 41,041 Da of the mature protein agrees with the apparent molecular mass determined by SDS-PAGE. There was no polyadenylation site in any clone.

Computer analysis of the predicted sequence indicated a high content of α-helical structure (Fig. 5); four possible amphipathic helices between residues 93 and 110, 140 and 152, 227 and 243, and 353 and 370 that may be responsible for lipid binding (Fig. 5); and one possible N-glycosylation site, consensus sequence N-X(S/T)N, at positions 246–249. There are three threonines (Thr-202, Thr-209, and Thr-292) and one serine (Ser-170) that are potential O-glycosylation sites. The search for homology with sequences in the SWISS-PROT and GenBank™ data bases did not show any significant similarity between apoL and any other known protein.

Apolipoprotein L Is Not Free in Plasma and Is Mainly Associated with Apolipoprotein A-I-containing Lipoproteins—We studied the distribution of apoL among apoA-I and apoB lipoproteins using selected affinity immunosorption. The experimental procedure is shown in Fig. 6A. Using successive anti-apoA-I and anti-apoB columns, we obtained the following fractions: lipoprotein-deficient plasma, apoA-I-containing lipoproteins (Lp(A-I)), and apoB-containing apoA-I-deficient lipoproteins (Lp(B w/o A-I)). Fig. 6B shows an immunoblot obtained after SDS-PAGE of 20 μg of protein from each. The 42-kDa apoL was present as a single reactive band in Lp(A-I). ApoL was undetectable in the Lp(B w/o A-I) and lipoprotein-deficient plasma fractions. This result suggests that apoL is associated chiefly with the apoA-I-containing lipoproteins.

Apolipoprotein L Is Present in a Dense HDL Fraction—the distribution was also compared among lipoproteins prepared by ultracentrifugation. VLDL, IDL, LDL, HDL2, and HDL3 were isolated by conventional sequential ultracentrifugation. Because this technique is known to disrupt lipoprotein structure (13–15, 33–35), we attempted to minimize lipoprotein alteration by submitting each lipoprotein class to equivalent ultracentrifugal forces as the v2t product, using the same rotor. Qualitative detection of apoL in the different subclasses was maximized by immunoblotting samples overloaded on SDS-polyacrylamide gel (100 μg of each fraction). Only minor amounts of apoL were detected in VLDL and HDL2. ApoL was primarily present in HDL3 (1.21–1.12 g/ml) (Fig. 7A). Because of the long exposure to the substrate, artifact bands (larger than apoL) were also revealed in IDL and LDL. The quantitative assay for apoL confirmed this by showing an apoL content in HDL of 2.60.7 μg of apoL/mg of total protein versus 0.13 ± 0.13 μg of apoL/mg of total protein in VLDL (Fig. 7B). ApoL was also detected in the bottom fraction (d > 1.25 g/ml) in trace amounts.

Immunosorption with an Anti-apolipoprotein L Affinity Gel—To find the subpopulation of Lp(A-I) containing apoL (Lp(A-L)), we constructed an anti-apoL column using purified anti-apoL IgG. We isolated apoL-containing lipoproteins (Lp(L)) directly from normolipidemic plasma. Fig. 8 shows an SDS gel comparing the bound fraction (Lp(L)) with the apoA-I-containing lipoproteins. By immunoblotting with specific antisera, we were able to detect the presence of apolipoproteins A-I, A-II, A-IV, and C-III (data not shown). Fractionation of
Lp(A-I) into the Lp(A-I:L) and Lp(A-I w/o L) fractions showed that only ~10% of Lp(A-I) contained apoL. Nondenaturing PAGE of these particles revealed two major Lp(L) subspecies based on their diameters (Fig. 9). ApoL was mainly distributed in large apoA-I-containing lipoproteins (12.2–17 and 10.4–12.2 nm) and was totally absent in the small particles. Moreover, the analysis of Lp(L) lipoproteins by immunoelectrophoresis revealed α- and pre-β-migrating components (Fig. 10).

**DISCUSSION**

We have reported here the identification, characterization, and cloning of a new human apolipoprotein that we have designated apolipoprotein L. This new apolipoprotein is mainly associated with the apoA-I-containing lipoproteins of plasma. High density lipoproteins comprise a number of molecular subspecies that differ with respect to protein and lipid composition, particle morphology, and size. The numerous HDL molecular species are not fully apparent when HDL is prepared by ultracentrifugation. Hydrostatic pressure developed in the ultracentrifuge causes the dissociation of a portion of the complement of apolipoproteins (such as apolipoproteins A-I, A-II, C, and E) from HDL and leads to concomitant protein and lipid rearrangements (13–15, 33–35). The contents of proteins such as lecithin:cholesterol acyltransferase and cholesterol ester transfer protein shown to interact and to form physical complexes with apoA-I-containing lipoproteins are diminished or totally depleted in HDL altered by ultracentrifugal isolation (15, 18). Thus, ultracentrifugation hinders identification of the molecular species of HDL and characterization of their constituent proteins.

Since first proposed by Alaupovic (16), numerous studies have shown the importance of immunoaffinity fractionation of lipoproteins. The development of the strategy of selected affinity immunosorption permits the isolation of native lipoprotein complexes with minimal perturbation (17), avoiding the loss of
protein constituents that dissociate during isolation by ultracentrifugation (13–15, 33–35). In this study, we combined liquid-phase isoelectric focusing and high resolution two-dimensional gel electrophoresis to surmount the problem posed by the predominance of apoA-I in the immunosolubilized Lp(A-I) fractions that would otherwise hinder purification of proteins present at lower concentrations.

ApoL isolated from the Lp(A-I) particles was observed in two forms: 42 and 39 kDa (minor form) (Fig. 1). The truncated species could represent a proteolytically activated form of the protein, as is the case for several other plasma apolipoproteins (36, 37). If so, the putative precursor form (42 kDa) represents the main constituent. So far, we have not been able to determine if this truncation occurs in vivo or during isolation.

Recently, Trofatter et al. (24) published an expressed sequence tag (clone C22-280, human chromosome 22) that matched the N-terminal sequence we had found for apoL. This sequence revealed 12 residues upstream of the first amino acid of the plasma form of apoL. Since this structure is typical of a signal peptide (38) and since the cDNA sequence of apoL reveals 12 residues upstream of the first amino acid for the mature protein of 371 amino acids with a molecular mass of 41,041 Da, in agreement with the experimental value, we propose that these 12 residues (starting with a methionine) represent the signal peptide of apoL. Therefore, the cDNA presented in this report encodes the full-length apoL protein.

The analysis of apoL cDNA (32) reveals one putative N-glycosylation site (N246SN249) and several candidate serine and threonine residues for O-glycosylation. Post-transcriptional modifications at these sites could explain the charge isoforms of apoL found in plasma (Fig. 1).

Because we did not find any significant homology between the apoL sequence and any present in SWISS-PROT or Genbank™ (22), it is not yet possible to predict any function of apoL based on homologies. However, the transcription of apoL mRNA by the pancreas suggests a very specific function, possibly enzymatic, in lipid metabolism. Indeed, preliminary data (not shown) seem to indicate a positive correlation between plasma levels of apoL and plasma triglyceride levels.

Analysis of the secondary structure of apoL (31) reveals four possible amphipathic helices (Fig. 5). These would confer a high level of lipophilicity, in agreement with our finding of very little detectable free apoL in plasma. That apoL in plasma is entirely bound to lipoproteins and remains associated with them during exposure to large volumes of buffer during column washing supports the view that it has very high affinity for HDL. Hence, it should be regarded as a true apolipoprotein rather than a plasma protein that exists partially in a lipoprotein-associated form such as haptoglobin. This is the basis of our designating it an apolipoprotein.

ApoL, with a mean plasma concentration of 5.9 ± 0.9 μg/ml (n = 5), is a marker for a distinct subpopulation of HDL. Indeed, apoL was found almost exclusively in association with apoA-I in lipoproteins prepared by immunoaffinity chromatography (Fig. 6). Moreover, the presence of apoL in plasma lipoproteins isolated by ultracentrifugation and its localization to HDL₃ (Fig. 7A) corroborate results obtained by immunoaffinity chromatography. Because of close association between apoA-I and apoL and because it is well known that lipoprotein integrity is better preserved by immunoaffinity isolation, we used the latter methodology to isolate specific lipoprotein subpopulations containing apoL. In agreement with our previous data, the apoL-containing lipoproteins (Lp(L)) contained apoA-I (Fig. 8). Moreover Lp(L) exhibited diameters typical of HDL (Fig. 9). However, it is interesting to note the discordance of the data between HDL purified by ultracentrifugation and the lipoprotein purified by immunoaffinity, showing the protein redistribution occurring during ultracentrifugation (13–15, 33–35). We found apoL to be preponderantly in HDL₃; however, the Lp(L) particles isolated by selected immunosorption exhibited heterogeneity of size. ApoL was chiefly associated with large HDL particles (Fig. 9). Fig. 9 also shows the existence of a very large apoL-containing lipoprotein corresponding to VLDL. Due to their low content in Lp(L) particles, these minor populations were not detectable by immunoblotting of apoB-containing lipoprotein (Fig. 7), but were only measurable by enzyme-linked immunosorbent assay. Fig. 7B shows the amount of apoL relative to the amount of total protein in the lipoprotein. The apoL content of HDL was >10 times higher than that in VLDL. No apoL was detectable in LDL. Apparently due to protein dissociation during ultracentrifugation, we also found apoL in the fraction of d > 1.25 g/ml. Moreover, apoA-II, apoA-IV, and apoC-III were also detected in Lp(L) (data not shown), indicating, as for other subclasses of HDL, the presence of a complex protein complement in Lp(L).

Populations of HDL designated as preβ-HDL have been postulated as serving key roles in reverse cholesterol transport (4, 6, 39). One, preβ₁-HDL, appears to act as the initial acceptor of cellular unesterified cholesterol (6). In this study, we showed that a subpopulation of apoL-containing lipoproteins also exhibits preβ mobility (Fig. 10), possibly belonging to the larger preβ₂ or preβ₃-HDL particle populations.

In summary, we have reported in this study the nucleotide and deduced amino acid sequences for a new human apolipoprotein that we have designated apolipoprotein L. This is the first apolipoprotein shown to be secreted by the pancreas. Its origin in that organ may reflect a non-insulin-dependent role of the pancreas in lipid metabolism. This new apolipoprotein is found in plasma, mainly associated with apoA-I-containing lipoproteins. Moreover, apoL-containing lipoproteins clearly define new HDL subtypes. Since no sequence homology was found with any known protein, its function cannot be inferred on a structural basis.

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