Human apoC-IV: isolation, characterization, and immunochemical quantification in plasma and plasma lipoproteins

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Abstract **Apolipoprotein C-IV (apoC-IV), the newest member of the low-molecular-weight apoC group, has been characterized in blood plasma of rabbits, in which it is a major proline-rich apoC component (Zhang, L-H., L. Kotite, and R. J. Havel. 1996. Identification, characterization, cloning, and expression of apoC-IV, a novel sialoglycoprotein of rabbit plasma lipoproteins. *J. Biol. Chem.* 271: 1776–1783). Although the decoded sequence of mouse and human apoC-IV is known, apoC-IV has not been identified in blood plasma from these or other species. Rabbit apoC-IV exists in several sialoforms, and the asialoform has an acidic isoelectric point. We show that apoC-IV is a basic protein in human, monkey, and mouse plasma, present as a minor apoC component of VLDL. Human apoC-IV, isolated from apo VLDL by DEAE-cellulose chromatography and two-dimensional electrophoresis, was identified by microsequencing four tryptic peptides. The protein exhibits two major isoforms; one is *N*-glycosylated, and both are variably sialylated. In normolipidemic plasma, greater than 80% of the protein is in VLDL (0.7% of total apo VLDL), with most of the remainder in HDL. The concentration of apoC-IV in the plasma and lipoproteins of $\rho < 1.21$ g/ml is closely related to plasma triglyceride concentration up to 1,770 mg/dl, varying from 0.1–1.9 mg/dl. Neither the human nor rabbit apoC-IV gene contains a typical TATA box in the 5′-flanking region, but the 5′-untranslated region of the rabbit gene contains a unique purine-rich sequence, GGGACAG(G/A), repeated nine times in tandem, with an additional two within the 5′-flanking sequence. This sequence, functioning as a GAGA box that has been implicated in the transcription of a number of genes, may explain the higher level of expression of apoC-IV in rabbits.**—Kotite, L., L-H. Zhang, Z. Yu, A. L. Burlingame, and R. J. Havel. **Human apoC-IV: isolation, characterization, and immunochemical quantification in plasma and plasma lipoproteins. *J. Lipid Res.* 2003.** 44: **1387–1394.**

Supplementary key words chromatography • electrophoresis • mass spectrometry • gene structure • 5′-untranslated region • apolipoprotein C-IV

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Apolipoprotein C-IV (apoC-IV) was first isolated from rabbit apo VLDL, in which it comprises a major component of total C apolipoproteins (1). ApoC-IV mRNA is expressed mainly or exclusively in rabbit liver, and the protein in plasma of chow-fed rabbits is distributed between VLDL and HDL, typical of C apolipoproteins. The decoded cDNA sequence of rabbit apoC-IV revealed an open reading frame encoding a protein of 124 residues with a typical signal peptide of 27 residues and a molecular mass of 11,020 Da. Owing to *O*-glycosylation, the apparent molecular weight of rabbit apoC-IV on denaturing gel electrophoresis is substantially higher. Variable sialylation with five isoforms is revealed by isoelectric focusing electrophoresis. Additional studies revealed a single major form of apoC-IV mRNA in liver, and a single copy of the apoC-IV gene per haploid rabbit genome.

Earlier work of others, in particular that of van Eck and associates (2), had identified a novel gene 5′ to the start site of the apoC2 gene in the apoE, apoC1, apoC2 gene cluster. The mouse gene that they identified encodes a protein of the precise length of rabbit apoC-IV with the same predicted amphipathic helical regions and striking sequence homology. As with the rabbit, mouse apoC-IV mRNA was expressed exclusively in liver. The sequence of the human apoC-IV gene, established subsequently by Allan and associates (3), encodes a protein that also bears close homology to that of mouse and rabbit apoC-IV, but with 127 rather than 124 amino acids, owing to a nine-nucleotide insert. The partial sequence of the monkey

Abbreviations: MALDI-MS, matrix-assisted laser desorption ionization mass spectrometry; NEPHGE, nonequilibrium pH gradient electrophoresis; PSD, post-source decay; SAA, serum amyloid A; 2-D, two-dimensional; UTR, untranslated region.

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gene determined earlier (4) also includes this insert. However, the overall organization of the mouse and human genes, as well as that of the rabbit (unpublished observations), is similar, with three exons and two introns. Allan and associates (3) found only a low level of expression of apoC-IV mRNA in human liver (about 1% of that of apoC-II), and they were unable to identify the protein in human blood plasma or isolated plasma lipoproteins by immunoblotting with a peptide antiserum (5). By immunoblotting with our antiserum to rabbit apoC-IV, we have found and report here evidence for the presence of this protein in apo VLDL from humans, Rhesus monkeys, and mice. We also report the isolation of apoC-IV from human apo VLDL and some of its properties. We have also developed a sensitive radioimmunoassay that has allowed us to quantify the concentration of apoC-IV in human plasma and ultracentrifugally separated lipoprotein fractions.

MATERIALS AND METHODS

Subjects

Blood samples were obtained from patients of the University of California, San Francisco Lipid Clinic who had fasted overnight. Protocols for blood drawing and use were approved by the Committee on Human Research of the University of California, San Francisco.

Antisera

Antiserum to rabbit apoC-IV, prepared in guinea pigs, has been described (1). Two antisera against human apoC-IV were raised, one against a synthetic peptide corresponding to predicted residues 28–38 of pro-apoC-IV (3) coupled to activated keyhole limpet hemocyanin (Pierce), and the other against highly purified asialo apoC-IV, prepared as described below. For each, rabbits were immunized by three injections of ~100 μg of the protein in complete Freund's adjuvant, initially with three booster injections of 50 μg each in incomplete Freund's adjuvant (Animals Services Pharmaceutical, Healdsburg, CA) over a 10 week period. Antisera were then collected and stored at −70°C. Antisera to human apoE (6), apoC-I (7) and amyloid A (Calbiochem-Novabiochem) were used to identify these proteins in Western blots of apo VLDL.

Gel electrophoresis

Rabbit apoC-IV is variably sialylated, thus expressing up to five isoforms in isoelectric focusing gel electropherograms (1). In preliminary experiments, an antiserum prepared in guinea pigs against rabbit apoC-IV recognized a protein component of human VLDL separated by isoelectric focusing electrophoresis with an apparent pI above 7. To characterize this component, VLDL from hypertriglyceridemic subjects was separated from fresh-blood plasma by ultracentrifugation (8) and then recentrifuged to remove contaminating plasma proteins. VLDL samples were delipidated in cold ethanol-ether (3:1, v/v). After centrifugation, the pellet was washed with ether (9), and then taken up into solubilizing buffer (0.01 M Tris-hydrochloride, pH 8.2, containing 6 M urea). Insoluble protein (apoB) was removed by brief centrifugation. Because the protein recognized by our antiserum appeared to be basic, we utilized a method (10) designed to resolve basic proteins [nonequilibrium pH gradient electrophoresis (NEPHGE)]. This method also employs ampholines, but electrophoresis is toward the cathode, with the acidic reservoir placed at the top and the basic reservoir at the bottom of the apparatus, opposite to that used for ordinary isoelectric focusing. To avoid collapse of the gradient at the basic end with consequent broadening of the protein spots, electrophoresis is restricted to <2,000 V-h; thus, basic proteins fail to reach their isoelectric points. We routinely used an apparatus (Hoefer) for 13 × 14 cm slab gels of 7% polyacrylamide containing l.5% ampholines, pH 5.0–10.5, in 7 M urea. To each lane of 1.0 cm, 50–100 μg of protein were applied and electrophoresis was carried out at 1,500 V-h at room temperature. Proteins were then transferred to nitrocellulose sheets (BioRad) by electroblotting (11) (Hoefer) and probed with anti-rabbit apoC-IV (1:400 dilution in 0.05 M Tris-hydrochloride and 0.15 M NaCl, pH 7.4, containing 0.05% Tween-20 and l% gelatin), anti-human apoC-I (1:2,000 dilution), and anti-human serum amyloid A (SAA) (1:2,000 dilution), followed by appropriate anti-IgG coupled to horseradish peroxidase. Peroxidase was detected by enhanced chemiluminescence (Western blotting detection reagent, Amersham). Because some components of human apoC-IV were found to cofocus with apoC-I and SAA, VLDL samples were alternatively treated with 0.1 unit of neuraminidase from Clostridium perfringens (Boehringer-Mannheim) per milligram protein in 0.02 M sodium acetate (pH 5.4) at 37°C for 2 h. NEPHGE was also utilized to identify immunoreactive protein in apo VLDL from a Rhesus monkey and C57Bl6 mice.

Isolation of human apoC-IV

For initial purification, soluble VLDL proteins (20–30 mg) were applied to a l × 10 cm column of diethyl-aminoethyl-cellulose (DE-52 Whatman) that had been prewashed with the solubilizing buffer. Under these conditions, apoC-I, together with other basic proteins, was excluded from the gel matrix and eluted in the void volume. Other proteins (E, C-II, and C-III) were eluted in a gradient of 0.01–0.15 M Tris hydrochloride (pH 8.2) containing 6 M urea. Only fractions from the void volume reacted with anti-rabbit apoC-IV. These fractions were pooled and concentrated 10-fold by dry dialysis against Sephadex G200 (Pharmacia).

To obtain highly purified apoC-IV, 100 μg of the void-volume fraction or 500 μg of total apolipoprotien VLDL (each desialylated or untreated) were subjected to two-dimensional (2-D) electrophoresis (12). For the first dimension, electrophoresis of the proteins in solubilizing buffer was carried out in tube gels (0.35 × 10 cm) in a nonequilibrium gradient of l.5–2% ampholines (pH 5.0–10.5), 2% Nonidet P-40, and 7 M urea in 3.75% acrylamide (37:l acrylamide-bis-acrylamide). Electrophoresis was at room temperature at 1,500 V-h. For the second dimension, electrophoresis was carried out in a 6–20% SDS polyacrylamide gradient gel. Proteins were probed with antisera as described above. To recover pure desialylated apoC-IV, the gels were briefly stained with 0.25% Coomassie blue R-250 in 40% methanol, 10% acetic acid, and electroeluted from stained gel pieces pooled from several gels. Protein content was estimated by the Lowry procedure (13) and by an ultrasensitive method (Nano-orange reagent, Molecular Probes, Inc.).

Determination of apoC-IV peptide sequences by mass spectrometry

Samples of desialylated apo VLDL, separated by 2-D electrophoresis, were briefly stained with Coomassie blue R-250 as described above. Gel pieces containing putative asialo-apoC-IV from 3–6 gels were diced into small pieces and placed into a microcentrifuge tube. The Coomassie blue stain was extracted by using a solution of acetonitrile-water (1:1, v/v) containing 25 mM ammonium bicarbonate. The gel pieces were covered with the solution and vortexed for 20 min and centrifuged briefly, then the blue supernatant was discarded. This process was re-

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peated several times until the gel pieces appeared white. The gel pieces were then dried under vacuum. In-gel digestion was performed by adding about 30 μl of trypsin solution (0.1 μg/μl) to reswell the gel pieces, and sufficient 25 mM ammonium bicarbonate to cover them. Digestion was carried out for 16 h at 37°C. Peptides in the digest solution were separated by HPLC, and the fractions were collected. The molecular weights of the peptides were determined by matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) (14). In order to obtain peptide sequence information, mass spectra were recorded under post-source decay (PSD) mode (15).

Quantification of apoC-IV in blood plasma and lipoprotein fractions

Blood from normolipidemic and hypertriglyceridemic subjects was collected into tubes containing disodium EDTA and benzamidine (final concentration 0.5 and 0.3 mg/ml, respectively) and placed on ice. Plasma, obtained after centrifugation (720 *g*, 30 min), was subjected to sequential ultracentrifugation at densities of 1.006, 1.019, 1.063, and 1.21 g/ml, or direct ultracentrifugation at a density of 1.21 g/ml (8). Fractions were dialyzed against 0.15 M NaCl containing 0.05% disodium EDTA (pH 7.2) and 0.02% sodium azide. Initially, we utilized the antiserum against residues 28–38 of pro-apoC-IV, which was reactive in Western blots (Fig. 3), in a dot-blot assay utilizing a microsample filtration manifold (Minifold I, Schleicher and Schuell). This assay yielded a linear response in the range of 10–100 ng asialo-apoC-IV and a clear signal with the protein in total lipoprotein samples ($\rho < 1.21$ g/ml). A more sensitive and precise radioimmunoassay was then developed to quantify apoC-IV in plasma and lipoprotein fractions, based upon an earlier procedure to quantify human apoE in which sodium decyl sulfate is used to maximize reactivity with antiserum (6). The peptide antiserum was unreactive in this assay, but the antiserum against isolated asialo-apoC-IV yielded maximal precipitation of radioiodinated apoC-IV in the presence of 2.7 mM sodium decyl sulfate in the range of 0.1–1 ng.

RESULTS

Identification of apoC-IV in human VLDL

Although the deduced amino acid sequence of human apoC-IV is highly homologous with that of rabbit apoC-IV (1, 3), that of the putative mature human protein contains more basic amino acid residues (eight lysine and eight arginine) and fewer acid residues (five glutamic acid and six aspartic acid), suggestive of a higher pI. Accordingly, we used NEPHGE as described in Materials and Methods to identify soluble proteins of human apo VLDL. By probing with anti-rabbit apoC-IV, multiple components were visualized, of which the two most prominent focused at pH 7.6 and 8.0 (**Fig. 1**). After treatment of apo VLDL with neuraminidase, the bulk of the immunoreactive protein focused at a pH close to 9 (Fig. 1). Immunoreactive apoC-I and isoforms of amyloid A were also identified by this procedure, but none of them focused above a pH of 8.5 (data not shown). The basic protein was thus provisionally identified as human apoC-IV.

By using a pH gradient of 5–10.5 in 2-D electrophoresis, two components of desialylated apo VLDL were visualized after staining with Coomassie blue R-250 (**Fig. 2**). One of these, with a predominant molecular weight of about

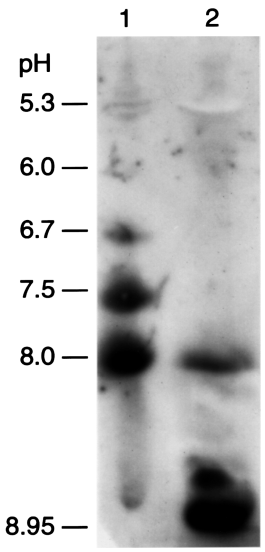

Fig. 1. Immunoblots of sialylated (lane 1) and desialylated (lane 2) human apo VLDL (300 μg protein each) separated by nonequilibrium pH gradient electrophoresis and transferred to a nitrocellulose sheet as described in Materials and Methods. The sheet was probed with guinea pig anti-rabbit apolipoprotein C-IV (apoC-IV) followed by ^{125}I-goat anti-guinea pig IgG.

37,000, was identified as apoE and the other, with an apparent pI near 9 and a predominant molecular weight of about 16,000, reacted with anti-human apoC-IV. A minor component with a molecular weight of about 21,000 was consistently evident. Components of slightly lower apparent molecular weights have been identified by SDS gel electrophoresis in mice transgenic for human apoC-IV (5). Similar results were obtained with the antiserum against the synthetic peptide corresponding to predicted residues 28–38 of human pro-apoC-IV (**Fig. 3**). With apo VLDL not treated with neuraminidase, several isoforms were evident similar to those observed in 1-D isoelectric focusing gels with anti-rabbit apoC-IV. The proportion of immunoreactive protein with higher molecular weight increased in isoforms with lower apparent pI, particularly at pH 6.8. After desialylation, the apparent pI of almost all of the two molecular weight species was close to 9, as observed with anti-rabbit apoC-IV. The intensity of staining with the human antiserum was stronger, however. The higher-molecular weight component in human apoC-IV transgenic mice was shown to be *N*-glycoslyated by Allan and associates (5); we have confirmed this observation for the protein in human VLDL (data not shown).

ApoC-IV in apo VLDL from other mammals

Apo VLDL prepared from blood plasma of a Rhesus monkey and pooled plasma of male mice was subjected to NEPHGE and probed with anti-rabbit apoC-IV as described above. Six immunoreactive components, with apparent pI similar to those of human apo VLDL, were observed with monkey apo VLDL. With mouse apo VLDL, four immunoreactive components were observed, also in the basic region. As indicated in **Table 1**, the apparent isoelectric point

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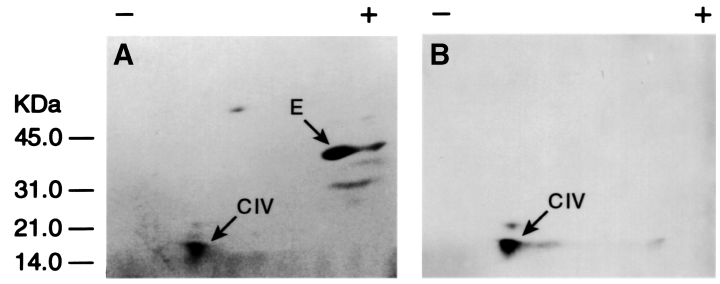

Fig. 2. Two-dimensional (2-D) electrophoretogram of 500 μg disialylated human apo VLDL. The gel was briefly stained in 0.25% Coomassie blue R-250 in 40% methanol, and 10% acetic acid (A). The proteins were transferred to a nitrocellulose sheet and probed with rabbit antiserum against a synthetic peptide corresponding to residues 28–38 of human pro-apoC-IV followed by anti-rabbit IgG coupled to horseradish peroxidase, and detected by enhanced chemiluminescence as described in Materials and Methods (B). A major band with apparent molecular mass of 16,000 kDa and a minor band of 21,000 kDa were evident both by staining and immunoreactivity. ApoE was identified with anti-human apoE (data not shown).

of the asialo form of the monkey and mouse proteins exceeded eight, similar to the human protein, and was distinct from the acidic isoelectric point of rabbit apoC-IV. The isoelectric points of human, rabbit, and mouse apoC-IV, predicted from the protein sequence of the mature protein, were in reasonable agreement with those observed.

Isolation of human apoC-IV

Initially, we separated basic soluble proteins from apo VLDL by chromatography on DEAE-cellulose prior to separation of apoC-IV by 2-D electrophoresis, as described in Materials and Methods. Subsequently, we were able to omit the chromatographic step by using larger amounts (up to 500 μg) of total apo VLDL directly. After brief staining with Coomassie blue and electroelution, both molecular weight components of putative apoC-IV could be identified in SDS gel electropherograms (**Fig. 4**). The major component of lower molecular weight was used for microsequencing and to obtain an antiserum in rabbits as described in Materials and Methods.

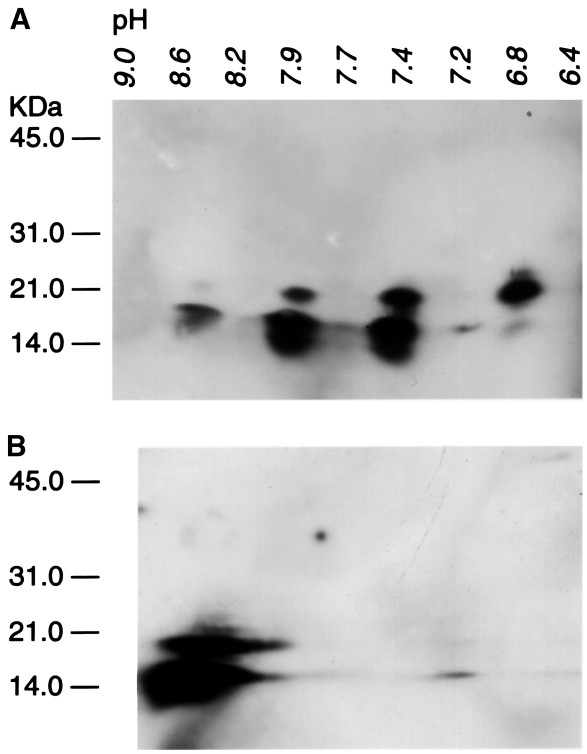

Fig. 3. Immunoblots of 450 μg human apo VLDL separated by 2-D electrophoresis and detected with peptide antiserum against human anti-C-IV, as in Fig 2. A: Sialylated apo VLDL. B: Desialylated apo VLDL. The minor component of molecular weight near 21,000 kDa is more highly sialylated than the major component of molecular weight near 16,000 kDa.

Sequencing of tryptic peptides

Molecular weights and sequences were obtained for four peptides by PSD mass spectrometry and MALDI-MS (**Fig. 5**). A total of 33 residues were thus identified, corresponding precisely to four sets of predicted sequences, one of which contained the predicted asparagine at residue 63 of pro-apoC-IV, the presumed site of *N*-glycosylation. No peptide containing the N-terminus of the mature protein was observed in several experiments.

Immunoassay of apoC-IV in plasma and lipoprotein fractions

The dot-blot assay yielded concentrations of apoC-IV in total lipoproteins of $\rho < 1.21$ g/ml in the range of 0.06–0.6 mg/dl. With the radioimmunoassay, displacement capacity was similar for VLDL and total lipoproteins of $\rho < 1.21$ g/ml, and the isotherms were parallel to those obtained with isolated asialo-apoC-IV (**Fig. 6**). The dot-blot

TABLE 1. Properties of apoC-IV from several mammals

Species	Number of Sialo Forms	Isoelectric Point (Asialo Form)	
	Observed	Predicted	Observed
Human	5	9.75	>8
Rhesus monkey	6	—	>8
Rabbit	5	5.04	5.7
Mouse	4	9.47	>8

Except for rabbit, focusing was by nonequilibrium pH gradient electrophoresis, which yields only approximate isoelectric points for basic proteins (see Materials and Methods).

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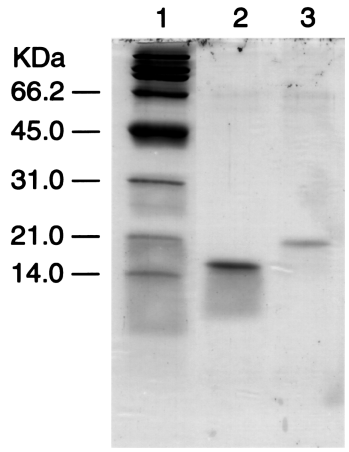

Fig. 4. SDS gel (12%) electrophoretograms of components of human desialylated apoC-IV electro-eluted from 2-D gels as described in Materials and Methods. Approximately 2 μg (lane 2) and 1 μg (lane 3) of the lower- and higher-molecular-weight species, respectively, were applied and stained with 0.25% Coomassie blue as described in Fig. 2. Lane 1: molecular weight markers.

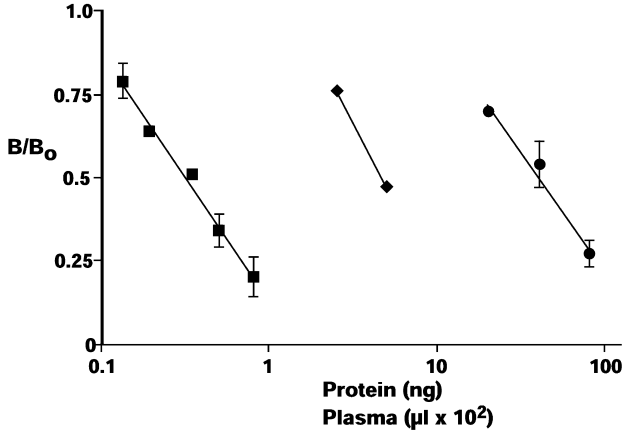

Fig. 6. Competitive displacement of ^{125}I-labeled human apoC-IV by unlabeled asialo-apoC-IV (squares, protein), apo VLDL (circles, protein), and a sample of whole human plasma (diamonds). Bars indicate SD for three preparations of asialo-apoC-IV and three preparations of apo VLDL (where not shown, bars fall within symbols).

and radioimmunoassays were compared in total lipoproteins from 19 samples of plasma, with triglyceride concentrations ranging from 69–1,162 mg/dl. The values were well correlated ($r = 0.80$), but the average concentration with the radioimmunossay was about 30% higher than with the dot-blot assay ($P < 0.02$ by paired *t*-test). With the sensitive radioimmunoassay, very little apoC-IV could be detected in proteins of $\rho > 1.21$ g/ml. In seven samples with varying triglyceride concentrations, apoC-IV was assayed in lipoproteins of $\rho < 1.21$ g/ml and residual proteins of $\rho > 1.21$ g/ml. A range of nil to 3.4% of total apoC-IV was found in the latter fractions (mean 1.5 ± 0.9%), independent of the concentration of plasma triglycerides. In five of these samples, the concentration of apoC-IV in whole plasma was compared with that in lipoproteins of $\rho < 1.21$ g/ml. Mean values were, on average, 11% higher in the latter fraction (not significant). These data indicate that apoC-IV is almost completely bound to plasma lipoproteins of $\rho < 1.21$ g/ml and can be measured in whole plasma by our radioimmunoassay.

The distribution of apoC-IV among plasma lipoprotein fractions was estimated in lipoproteins from three subjects with plasma triglycerides concentrations below 300 mg/dl. On average, 82% was in VLDL, and most of the remainder was in HDL. As expected from this distribution, the concentration of apoC-IV was highly correlated ($r = 0.77$) with that of plasma triglycerides (**Fig. 7**). Absolute values ranged from 0.06 to 1.09 mg/dl. ApoC-IV was a minor protein component, on the order of 1% or less, in VLDL subjected to a second ultracentrifugation to remove small amounts of albumin (range 0.29–1.26%, n = 13). The percentage tended to decrease with increasing triglyceride concentrations ($r = -0.43$, $P = 0.13$).

MSLLRNRLQALPALCLCVLVLACIGAC	27
QPEAQEGTLSPPPKLKMSR**WSLVR**GRMK	55
ELLETVVNRTRDGWQWP**WSPSTFR**	79
GFMQTYYDDHLRLLGPLTKAWFLESKD	106
SLLKKTHSLCPRLVCGDKDQG	127

Fig. 5. Predicted amino acid sequence of human pro-apoC-IV, with the sequences obtained for four tryptic peptides by matrix-assisted laser desorption ionization mass spectrometry shown in bold. The single asparagine at residue 63, the putative site of *N*-glycosylation, is underlined.

DISCUSSION

We have shown that apoC-IV is present in human plasma lipoproteins. Although its concentration is much lower than that of other C apolipoproteins, it can be regularly detected and quantified in plasma and lipoprotein fractions by our sensitive radioimmunoassay. Like other apoCs, it is almost exclusively bound to lipoproteins, mainly VLDL and HDL. However, in normotriglyceridemic plasma, apoC-IV is found predominantly (about 80%) in VLDL, whereas apoC-II and apoC-III are about equally distributed between VLDL and HDL (16, 17), and apoC-I is found predominantly in HDL (7, 18). As expected from the preferential association of apoC-IV with VLDL, its concentration in plasma is a function of VLDL and plasma triglyceride levels.

Why has apoC-IV escaped detection for so long? In humans and most other mammals, the low-molecular-weight protein components of triglyceride-rich lipoproteins (chylomicrons and VLDL) seem to be composed almost entirely of apoC-I, apoC-II, and apoC-III (19, 20). Relatively little attention, however, has been given to these proteins in mammals other than rodents and humans. Although we had observed an unusually complex pattern of soluble proteins in rabbit apo VLDL separated by isoelectric focusing

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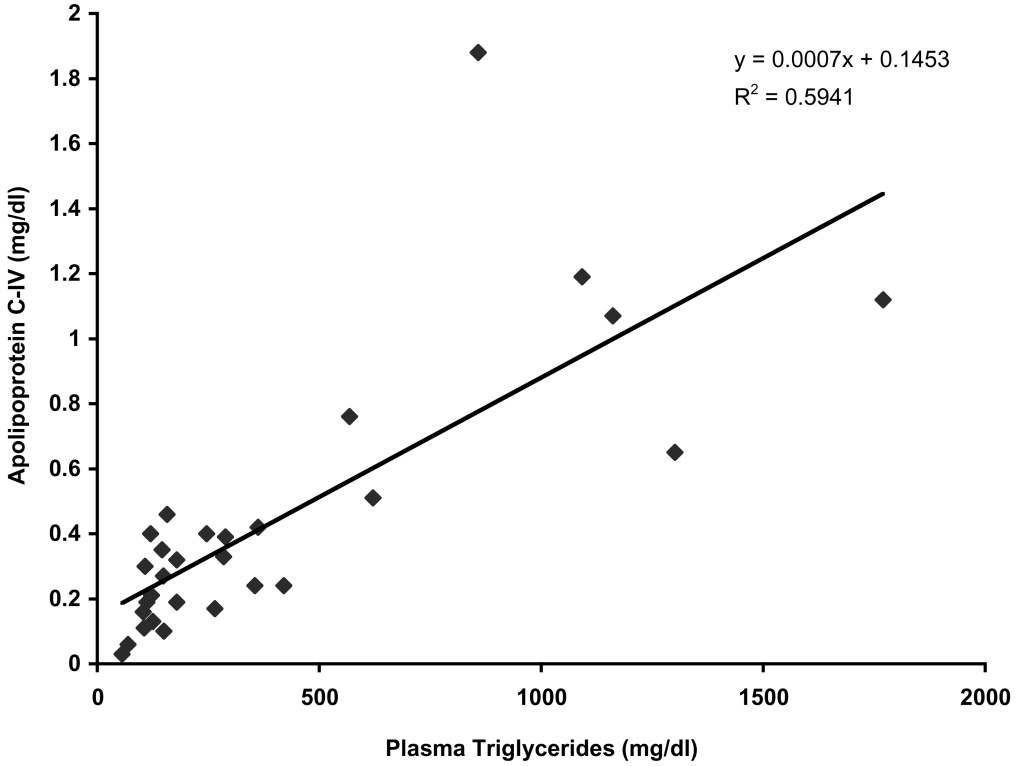

Fig. 7. Relationship of concentration of apoC-IV in $\rho < 1.21$ g/ml fraction of 29 samples of human blood to that of plasma TG. The regression line for the relationship is indicated together with the least-squares regression equation.

gel electrophoresis 20 years ago (21), it was many years later that we isolated and characterized rabbit apoC-IV as a major component of C apolipoproteins in this species (1).

The low prevalence of apoC-IV among human apoCs provides a partial explanation for the failure to detect it until now. In addition, however, basic proteins are poorly resolved under the usual conditions of isoelectric focusing electrophoresis. The higher pI of human apoC-IV is presumably explained by a relative preponderance of basic over acidic amino acid residues in its sequence. Although apoC-IV shares with apoC-III the presence of several variably sialylated isoforms, the human protein, as shown earlier by Allan and Taylor in their studies of human apoC-IV transgenic mice (5), is *N*- as well as *O*-glycosylated. Their data suggested that only the *N*-glycosylated form of the protein is sialylated. We find that both forms are sialylated, although the *N*-glycoslyated form has, on average, a greater number of sialyl residues (Fig. 3). Thus, human apoC-IV exhibits the greatest structural complexity of all the C apolipoproteins. Rhesus monkey apoC-IV presumably displays similar isoelectric complexity. Whereas mouse and rabbit apoC-IV lack asparagine, they display similar variability of sialylation (Table 1).

The concentration of apoC-IV in blood plasma of normolipidemic humans (~0.1 mg/dl) is approximately one order of magnitude lower than that of normolipidemic rabbits (1). That may reflect primarily the lower expression of the apoc4 gene in human as opposed to rabbit liver (1, 3). The structure of the rabbit apoc4 gene (see Appendix 1) closely resembles that of humans (3), each containing three exons: exon 1 encodes amino acids 1–25, exon 2 encodes amino acids 26–73 in humans and 26–70 in rabbits, and exon 3 encodes the remaining 73 amino acids. Neither gene contains a typical TATA box in the 5′-flanking region (see Appendix 1), and several SP1 binding sites (GC box) are spread through the 5′-flanking region and in intron 1. Also, as in humans (3), the rabbit gene starts at multiple sites, consistent with lack of a TATA box. Comparison of the 5′-untranslated region (UTR) of the two species, however, shows a unique purine-rich sequence, GGGACAG(G/A), in the rabbit apoC4 gene, repeated nine times in tandem (see Appendix 2) with an additional two within the 5′-flanking sequence. By contrast, the human and mouse apoC4 genes each contain only a single GGGACAG(G/A) repeat. This type of purine-rich sequence appears to be a GAGA-like box, which has been implicated in activation of transcription of a number of genes (22), including osteonectin (23) and rat insulin I (22). Furthermore, a purine-rich sequence, AGAGAGAGGGAGAGAGGAGCCGAGAGGGAGAGGGAGAGGGAGAG, called the raloxifene-response element, in the 5′-UTR of the human gene for transforming growth factor β-3, has been found to be involved in mediating its activation by 17-β estradiol and its metabolites (24). The GGGACA sequence is also found in the glucocorticoid regulatory region of rat α-2μ-globulin (25). Therefore, the uniquely long GGGACAG(G/A) repeats in the rabbit apoC4 gene may function as an important polypurine-response element for factors involved in its transcriptional activation or the stability of its mRNA.

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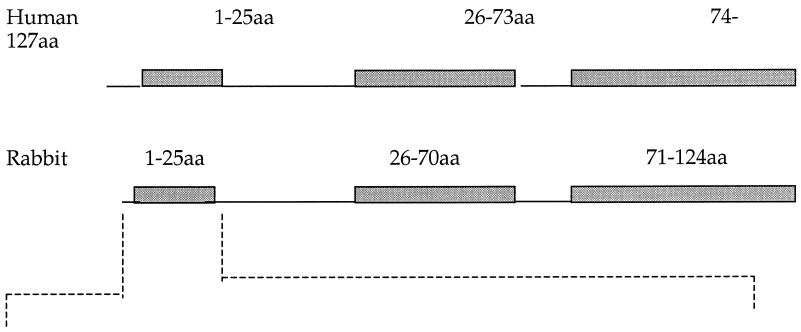
As we noted earlier, the conservation of the genomic and protein structure of apoC-IV among rodents, lagomorphs, and primates suggests that apoC-IV has an important function in lipoprotein metabolism (1). Overexpression of human apoC-IV in mice to yield an estimated concentration of 5–10 mg/dl in blood plasma produced a hypertriglyceridemic phenotype (5), as found with overexpression of other apoCs in this species. Recently, polymorphisms in the human apoC4 gene, Leu36Pro and Leu96Arg, have been found to be associated with plasma triglyceride levels in women (26), further suggesting that apoC-IV has a role in regulation of lipoprotein-lipid metabolism.

APPENDIX 1

Genomic structure and sequence of the 5′-region of the rabbit apoc4 gene

Schematic comparison of the human and rabbit apoc4 genes.



Sequence of the 5' region of the rabbit apoc4 gene: small letters show partial 5'-flanking or intron 1 sequence; large letters show exon l, and italics show GGGACAG(A/G) repeats.

CAT-box? Sp1

ATTCGCAGAGA*GGGACAGAGGGACAGA*CAGAGATTTTCTATCTC*CCAAT*TCACTCCCTA

AATGTTCACAACGTGTGGGTCTGGAACAGGCCAAAGCCAGGAACTCCATCCAGGTCTCCCACT

TGGGTAACAGGGACCCAAGTAGTTGAGCCATCACCTGCTCACCCAGAATTCGGCCGAATTC

Exon 1

GCAGAGA***GGGACAGAGGGACAGAGGGACAGAGGGACAGGGG G***

ACAGAGGGACAGGGGGACAGAGGGACAGGGGGACAGGGGG

AAGAAGCCCCCAGAA**ATG**CTGCTCCCGAGACGCGGGCTCCG

Sp1

GACCCTGCCCTCCCTCTGCCTCTACATCCTGGTCCTGGTCTG

Intron 1

Sp1 Sp1

GGTCGTGGGTGAGGAGGGGGTGAGGGCTCTGGAGGGGCGGCTTGGGCACTTGGTGG CC

TCCGTGTGTGCTGGGTGGTGGGAGGCGGCTGTGTCAGGGGCTGAGTA

APPENDIX 2

Comparison in three species of 5′-untranslated region of apoC-IV gene

```
Human      1 ----------------------------------------------------GAGTTGAGC
Mouse      1 ----------------------------------------------------GAGTACAGC
Rabbit     1GCAGAGAGGGACAGAGGGACAGAGGGACAGAGGGACAGGGGGACAGAGGGACAGGGGAC

Human     10 A-------CAGAGGGACAGAGGCACGGAACCCCCAGAAATGTCCC
Mouse     10 AGGAGGGACAGAGATAGAGAGGGACCAAGTCCCCAGAAATGTCCC
Rabbit    61 AG-AGGGACAGGGGGACAGGGGAAGAAGCCCCCAGAAATGCTGC
```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