Effect of apolipoprotein C-I peptides on the apolipoprotein E content and receptor-binding properties of beta-migrating very low density lipoproteins

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Abstract To evaluate the role of apolipoprotein (apo) C-I in inhibiting lipoprotein binding to the low density lipoprotein receptor-related protein (LRP), a putative lipoprotein remnant receptor, apoC-peptide fragments were prepared by chemical synthesis or by cyanogen bromide cleavage of intact apoC-I. In ligand-blotting assays, peptides corresponding to residues 1–38, 10–57, 20–57, 30–57, and 40–57 proved ineffective, but intact apoC-I was very effective, at inhibiting the binding of apoE-enriched β-migrating very low density lipoproteins (β-VLDL) to the LRP. Studies of the displacement of ^{125}I-labeled apoE from apoE-enriched β-VLDL showed that the largest peptide (residues 10–57) was two-thirds as effective as intact apoC-I; the other peptides were highly ineffective (residues 40–57, 1–38) or only partly effective (residues 20–57, 30–57). Changes in the intrinsic tryptophan fluorescence and helix content indicated that the largest peptide was similar to apoC-I in lipid binding affinity, while the other peptide fragments showed little or no affinity for either unilamellar or multilamellar vesicles of dimyristoylphosphatidylcholine. These findings suggest that the ability of apoC-I fragments to displace apoE from β-VLDL is largely, but perhaps not exclusively, a reflection of their ability to bind to membranous bilayers and that apoC-I blocking of the interaction between apoE-rich β-VLDL and the LRP probably involves displacement of a critical amount of the apoE from the surface of this lipoprotein.—**Swaney, J. B., and K. H. Weisgraber.** Effect of apolipoprotein C-I peptides on the apolipoprotein E content and receptor-binding properties of beta-migrating very low density lipoproteins. *J. Lipid Res.* 1994. **35:** 134–142.

Supplementary key words ligand blotting • peptide synthesis

To investigate the action of apolipoprotein (apo) C-I in modulating β-migrating very low density lipoprotein (β-VLDL) binding in the liver, studies were undertaken to determine whether apoC-I fragments might be as competent as the intact form in displacing apoE from β-VLDL or preventing it from binding to the low density lipoprotein receptor-related protein (LRP). The C apolipoproteins have previously been shown to play an important role in lipoprotein metabolism through their ability to transfer between lipoprotein classes, particularly between high density lipoprotein (HDL) and chylomicrons, and, in so doing, to alter the properties of these particles (1, 2). ApoC-II, for example, appears to be required for lipolysis of triglyceride-rich lipoproteins by lipoprotein lipase and is known to activate this enzyme (3). Similarly, apoC-I has been shown to activate the enzyme lecithin:cholesterol acyltransferase (LCAT), although this ability is shared and even surpassed by other apolipoproteins, most notably apoA-I (4, 5). The presence of C apolipoproteins on the chylomicron surface has long been associated with delayed clearance of these particles from the circulation, while their loss from the chylomicron surface during lipolysis and remnant formation is correlated with an enhanced rate of uptake by the liver (1, 6, 7). Studies involving the addition of purified C apolipoproteins to native and model chylomicrons have implicated both apoC-III and apoC-I as major determinants of chylomicron uptake by the liver, but their mechanism of action remains obscure (8, 9).

Removal of chylomicron remnants from the circulation appears to involve both the classical LDL receptor and a specific chylomicron remnant receptor (10). The LDL receptor-related protein has been postulated to be the chylomicron remnant receptor, and binding to this receptor has been shown to be mediated by apoE (11, 12) as well

Abbreviations: apo, apolipoprotein; CD, circular dichroism; HPLC, high pressure liquid chromatography; HDL, high density lipoprotein; LCAT, lecithin:cholesterol acyltransferase; LDL, low density lipoprotein; LRP, LDL receptor-related protein; PVDF, polyvinylidene difluoride; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; UV, ultraviolet; VLDL, very low density lipoprotein; DMPC, dimyristoylphosphatidylcholine.

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as by lipoprotein lipase (13). The lipoprotein most widely used for studying the binding to the LRP is a unique species of very low density lipoproteins (VLDL) known as β-VLDL (14). β-VLDL, a major class of lipoproteins in cholesterol-fed animals, consist of a mixture of remnants derived from chylomicrons and VLDL (15). Studies by Windler and Havel (1) and by Quarfordt, Michalopoulos, and Schirmer (16) showed that addition of C apolipoproteins inhibited triglyceride-rich lipoprotein uptake by perfused livers. Subsequently, addition of pooled C apolipoproteins was shown to prevent binding of apoE-enriched β-VLDL to the LRP (17). Recently, Weisgraber and colleagues (18) demonstrated that this inhibitory activity resided principally in apoC-I, and that potency of the C apolipoproteins to inhibit β-VLDL binding to the LRP correlated with their ability to displace apoE from the lipoprotein. Alternatively, apoE could exist in conformations that were both competent or noncompetent with respect to receptor binding (19–21), and interaction with apoC-I could favor a conformation of apoE on the β-VLDL which is incapable of binding to its receptor. Such a mechanism was proposed by Windler and Havel (1), based on their studies of the effects of rat and human C apolipoproteins on remnant uptake by perfused rat livers; Sehayek and Eisenberg (22) have also concluded that their data are consistent with this hypothesis.

For a further investigation of the possible role of protein–protein interactions between apoC-I and apoE in lipoprotein complexes, fragments of apoC-I were prepared in order to determine whether the ability of polypeptides to inhibit binding of β-VLDL to LRP could be correlated with the ability of these fragments to displace apoE from β-VLDL and to establish whether apoE displacement is a reflection of relative affinities for phospholipid bilayers. Previous studies of synthetic peptides corresponding to segments of the primary structure of apoC-I suggested that the lipid binding capacity of apoC-I, a polypeptide of 57 residues, appears to reside in the carboxyl-terminal half of the molecule, while the ability to activate LCAT required the carboxyl-terminal 41 residues (23). Peptide fragments of apoC-I used in this study included similar regions, allowing us to compare newly described functional properties of apoC-I with previously established functions localized to particular regions.

MATERIALS AND METHODS

Lipoproteins

The β-VLDL were prepared from the plasma of rabbits fed for 4 days with a 2% (w/v) cholesterol, 10% (v/v) coconut oil diet as described (17) and were then dialyzed against 0.15 M NaCl and 20 mM sodium phosphate (pH 7.4). This fraction was successively filtered through 5-μm, 1.5-μm, 0.8-μm, and 0.45-μm filters as described by Kowal et al. (17). In one set of experiments the β-VLDL was provided as a gift from Dr. J. Herz of the University of Texas Southwestern Medical Center at Dallas. For most experiments the β-VLDL was biotinylated as described by Kowal et al. (17), except that biotin-LC-hydrazide (Pierce Chemical Co.) was used in lieu of biotin hydrazide. After biotinylation, the β-VLDL was dialyzed against 20 mM Tris-HCl, 0.15 M NaCl, 0.25 mM EDTA, pH 7.5.

Apolipoproteins

Human apolipoproteins C-I, C-II, C-III-1, and C-III-2 were isolated from the low molecular weight peak obtained during gel filtration on Sephacryl S-300 of the d < 1.02 g/ml lipoprotein fraction and by preparative high pressure liquid chromatography (HPLC) on a 2.15 × 15 cm DEAE 5PW column (Bio-Rad) as previously described (18). Lyophilized fractions of pure C apolipoproteins were renatured by solubilization in 6 M guanidine HCl and slow removal of the denaturant by dialysis against 10 mM ammonium bicarbonate. Rabbit plasma apoE was isolated using gel filtration on Sephacryl S-300 (Pharmacia LKB Biotechnology Inc.). This material was stored at −70°C in 0.1 M NH_4HCO_3 and renatured from guanidine HCl as described above.

Synthetic peptides

Various-length fragments of human apoC-I coterminating at the apoC-I carboxyl terminus were synthesized using an Applied Biosystems model 431A Peptide Synthesizer. The standard program was modified slightly by adding a capping step with acetic anhydride to block unreacted amino termini. All but two cycles of the synthesis yielded an efficiency >98.5% for the coupling of the protected amino acid to the growing polypeptide chain. At predetermined points in the synthesis, portions of the peptide-resin complex were removed and the remainder was returned to the synthesizer to continue the synthesis, allowing for a "nested" synthesis of fragments C-I_{40-57},[2] C-I_{30-57}, C-I_{20-57}, and C-I_{10-57}. After deprotection and cleavage from the resin, each peptide was analyzed and purified by reversed phase HPLC on a C8 column (Beckman Ultrasphere ODS, 4.6 × 250 mm). The purity of each peak was ascertained by UV absorbance spectroscopy, rechromatography on a C8 column, amino acid analysis, and polyacrylamide gel electrophoresis in gels containing sodium dodecyl sulfate (SDS-PAGE) (24). The peptides were stored in the lyophilized form and reconstituted before use by dissolving in 6 M guanidine HCl and passing over a small gel filtration column (Bio-Gel P-2, Bio-Rad Labs) which was eluted with 10 mM ammonium bicarbonate.

[2]The peptide fragments of apoC-I are referred to by the expression C-I_{a-b}, where *a* and *b* refer to the beginning and ending residues in the primary structure, respectively.

Cyanogen bromide cleavage-generated peptide fragments

Apolipoprotein C-I was cleaved at the single methionine residue (residue 38) by incubation overnight with a 100-fold excess of CNBr in 70% formic acid. After lyophilization, the peptide fragments were resolved by reversed phase HPLC on a C8 column. The peptide corresponding to residues 1–38 ($C\text{-}I_{1\text{-}38}$) was characterized by ultraviolet absorption spectroscopy, SDS-PAGE, and amino acid analysis.

Determination of ^{125}I-labeled apolipoprotein E bound to β-VLDL

Rabbit apoE was iodinated with Bolton-Hunter reagent (DuPont-New England Nuclear) as described (25) (specific activities of the two preparations used in these studies were 1600 and 554 dpm/μg). Incubation mixtures contained 50 μg of biotinylated β-VLDL protein, 100 μg of ^{125}I-labeled rabbit apoE, 1% β-mercaptoethanol, and 0–100 μg of apoCs or apoC-I synthetic fragments. A constant final volume was obtained by adding 0.1 M NH_4HCO_3, and 100 μl was applied to a Superose-12 fast-performance liquid chromatography (FPLC) column, 10 × 300 mm (Pharmacia). The column was eluted with 0.15 M NaCl, 20 mM sodium phosphate, 1 mM EDTA (pH 7.4) at a flow rate of 1 ml/min, and fractions were collected at 30-sec intervals.

Ligand blotting

Ligand blotting of the LRP and LDL receptors was performed by electrophoresis of a DEAE cellulose fraction of rat liver membranes (17) on 3–10% SDS-PAGE gels, electroblotted overnight at 250 ma onto polyvinylidene difluoride (PVDF) membranes (Immobilon, Millipore). The membrane was blocked for 1 h with a 5% bovine serum albumin solution. The membrane was cut into 3–4 mm strips, which were incubated for 1 h at room temperature with β-VLDL (10 μg protein/ml) that had been preincubated at 37°C for 30 min with rabbit apoE (10 or 20 μg) and apoC-I or synthetic fragments. After washing the membrane strips (3 × 10 min washes), the bound biotinylated β-VLDL was "decorated" by incubating the strips with ^{125}I-labeled streptavidin, which was prepared by radioiodinating streptavidin (Pierce) using Iodo-Gen (Pierce) as a catalyst. The specific activity of this material was ~7000 dpm/ng; autoradiograms were prepared using XM film (3M Company) using a cassette with intensifying screens.

Circular dichroism

The alpha helical content of lipid-bound and lipid-free peptides was determined by measuring the circular dichroism (CD) spectrum over the interval of 200–230 nm using a Jasco J41A spectropolarimeter calibrated with a 0.1% (w/v) *d*-10-camphorsulfonic acid solution. Lipid complexes were prepared with peptides freshly renatured from 6 M guanidine HCl by incubation with unilamellar vesicles of dimyristoylphosphatidylcholine (DMPC) (DMPC–peptide, 2.5:1, w/w) at 24°C for 1 h; the samples were stored at 4°C overnight and spectra were obtained the next day. CD spectra were measured at room temperature in a 0.1-cm path length quartz cell at a protein concentration of 50 μg/ml in 10 mM sodium phosphate buffer, pH 7.6. An average was taken over 12 scans, and the ellipticities at 222 nm and 208 nm were used to estimate the percentage of α-helix, by the method of Greenfield and Fasman (26).

Fluorescence spectroscopy

The lipid binding characteristics of apoC-I and its peptide fragments were evaluated by titrating the effect of serial additions of phospholipid on the intrinsic fluorescence of the single tryptophan residue. A 1 mg/ml solution of DMPC was freshly prepared by sonication to yield a clear solution of unilamellar vesicles. Aliquots of this solution were added to a cuvette (which was placed in a thermostated holder) containing the peptide and allowed to incubate 5–10 min at 24°C before obtaining the fluorescence emission spectrum from 300–400 by excitation at 290 nm in a Hitachi F-2000 fluorescence spectrophotometer. SpectraCalc software was used to process the spectra and to determine the emission wavelength maximum. In another series of experiments, multilamellar dispersions of DMPC, prepared at a concentration of 75 μg/ml, were placed in a thermostated fluorescence cuvette; an aliquot of peptide calculated to yield a DMPC:peptide ratio of 1.5:1 (w/w) was added, and the clearing of phospholipid turbidity was followed over 30 min to evaluate the interaction between these two chemical species.

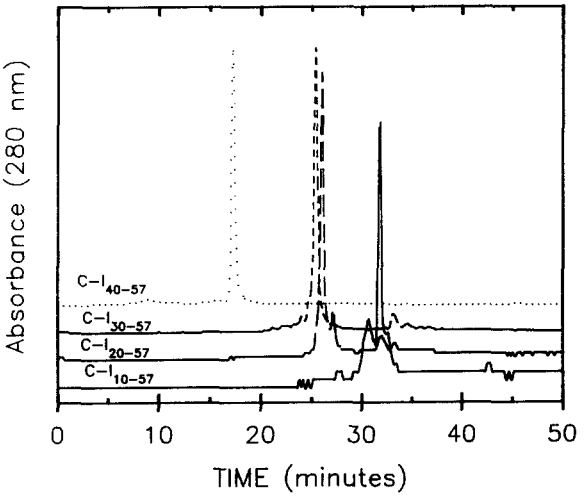

Fig. 1. Reversed phase HPLC of pooled fractions from preparative-scale HPLC of synthetic peptides. Peptide fractions from the nested synthesis of apoC-I carboxyl-terminal fragments were injected onto a 10 × 150 mm C_8 reversed phase column and eluted with a gradient from 25% to 60% acetonitrile.

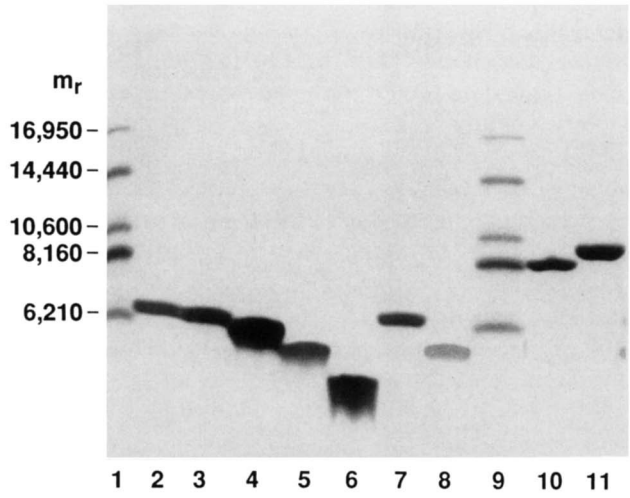

Fig. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of C apolipoproteins and peptide fragments of apolipoprotein C-I. Samples (5–8 μg) were applied to each of the following lanes: lanes 1 and 9, molecular weight markers (Sigma, MW-SDS-17S), lanes 2 and 7, apoC-I; lane 3, C-I_{10-57}; lane 4, C-I_{20-57}; lane 5, C-I_{30-57}; lane 6, C-I_{40-57}; lane 8, C-I_{1-38}; lane 10, apoC-II; lane 11, apoC-III-1.

RESULTS

Solid-phase peptide synthesis of apoC-I fragments, followed by cleavage and deprotection, yielded preparations which were subjected to preparative reversed phase HPLC. The major fractions were pooled and characterized by ultraviolet spectroscopy, after which pooled fractions were subjected to analytical reversed phase HPLC (**Fig. 1**). Each of the four peptides revealed a single major peak, with the longer peptides eluting later as expected. As apoC-I possesses no tyrosine residues and only a single tryptophan (residue 41), all peptides yielded identical UV spectra in the aromatic region of 260–290 nm.

Reversed phase HPLC was used to isolate and purify the amino-terminal cyanogen bromide fragment of apoC-I, corresponding to residues 1–38. The purity of this peptide, as well as of the synthetic fragments, was > 90%, as determined by SDS-PAGE (**Fig. 2**). The molecular weights for each synthetic fragment and for the cyanogen bromide fragment were in the expected ranges. Amino acid analysis of the peptides (**Table 1**) showed good agreement with expected values.

The ability of peptides to displace apoE from the surface of β-VLDL was assessed via a gel filtration assay utilizing a Superose 12 column. A sample of β-VLDL incubated with ^{125}I-labeled apoE plus various amounts of peptides was applied to the column to separate the β-VLDL from unbound apolipoproteins. As shown in **Fig. 3,** displacement of apoE from the β-VLDL to a later-eluting fraction was dependent upon the amount of peptide added; the effect of the fragments of apoC-I diminished as the length of the peptide decreased. To compare the properties of the peptide fragments quantitatively, the relative displacement of apoE was normalized to the control incubation (no apoC peptide added) at 100% (**Fig. 4**). As shown in Fig. 4A, the longest fragment (C-I_{10-57}) comes closest to duplicating the ability of intact apoC-I to displace apoE. Shorter peptides (C-I_{20-57} and C-I_{30-57}) were less effective in displacing apoE, behaving similarly to apoC-III (Fig. 4B). Neither the shortest fragment (C-I_{40-57}, Fig. 4A) nor the C-I_{1-38} fragment (Fig. 4B) appeared to compete with apoE for the surface of β-VLDL.

TABLE 1. Amino acid composition of purified synthetic fragments of apolipoprotein C-I

Amino Acid	C-I_{1-38} [a]	C-I_{40-57} [b]	C-I_{30-57} [a]	C-I_{20-57} [a]	C-I_{10-57} [a]
Asx	2.9 (4)[c]	1.0 (1)	1.2 (1)	2.1 (2)	2.9 (3)
Glx	4.9 (5)	4.2 (4)	6.5 (6)	8.5 (7)	10.0 (9)
Ser	4.8 (5)	1.6 (2)	3.4 (4)	4.0 (5)	3.8 (5)
Gly	1.1 (1)	0.0 (0)	0.0 (0)	0.0 (0)	0.87 (1)
His	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Arg	2.3 (2)	0.0 (0)	0.94 (1)	2.6 (3)	3.1 (3)
Thr	1.9 (2)	0.80 (1)	0.84 (1)	0.87 (1)	1.8 (2)
Ala	3.2 (3)	0.0 (0)	0.85 (1)	1.6 (2)	1.7 (2)
Pro	0.76 (1)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Tyr	0.23 (0)	0.05 (0)	0.20 (0)	0.24 (0)	0.21 (0)
Val	1.1 (1)	1.0 (1)	1.1 (1)	1.2 (1)	1.2 (1)
Met	0.09 (0)	0.70 (1)	1.1 (1)	1.1 (1)	1.2 (1)
Cys/2	0.06 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Ileu	1.9 (2)	1.1 (1)	1.1 (1)	2.7 (3)	3.1 (3)
Leu	5.2 (5)	1.2 (1)	2.1 (2)	3.1 (3)	5.2 (5)
Phe	1.9 (1)	2.1 (2)	2.1 (2)	2.4 (2)	3.1 (2)
Lys	5.6 (5)	4.0 (4)	6.4 (6)	7.1 (7)	9.5 (9)
HomoSer	0.71 (1)				

[a] Average of three determinations.
[b] Average of two determinations.
[c] Average moles amino acid/mole peptide; the numbers in parentheses are the expected values.

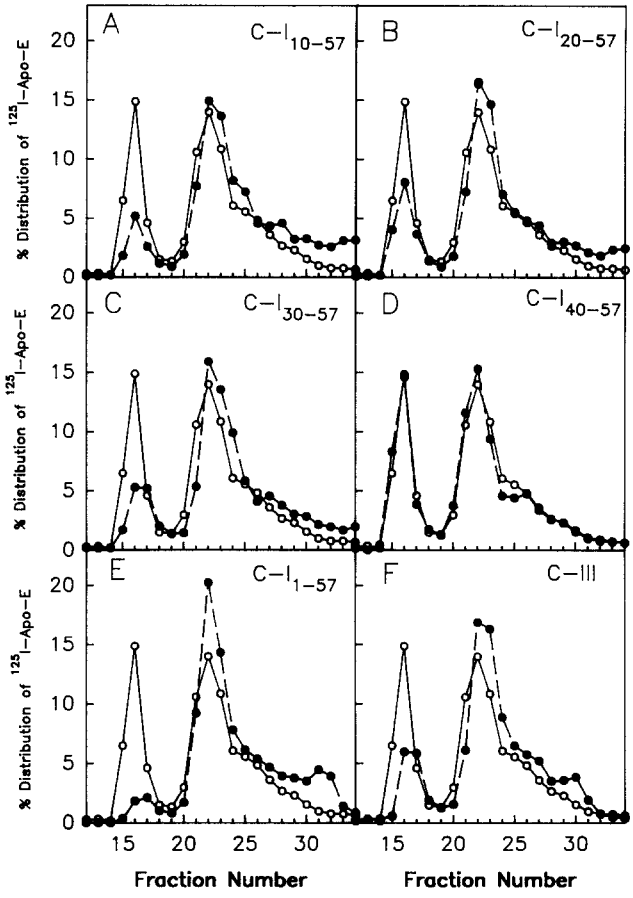

Fig. 3. Displacement of ^{125}I-labeled apoE from β-VLDL by various concentrations of apolipoprotein C-I fragments as determined by gel filtration. A mixture of β-VLDL (50 μg protein) and ^{125}I-labeled apoE (100 μg) was incubated at 37°C for 30 min with increasing amounts of apoC-I fragments (final volume = 300 μl). A 10-μl aliquot of the mixture was applied to a Superose 12 column. The column was eluted with 0.15 M NaCl, 20 mM sodium phosphate, 1 mM EDTA (pH 7.4) at a flow rate of 1 ml/min, and fractions were collected at 30-sec intervals. The percent distribution of radioactivity was determined by γ counting. Fractions 15–18 contained β-VLDL, and fractions 20–32 contained free apoE. Panels A-F correspond to the addition to the incubation mixture of the following peptides, respectively: C-I_{10-57}, C-I_{20-57}, C-I_{30-57}, C-I_{40-57}, C-I_{1-57}, C-III; (○), control (no additions); (●), 100 μg peptide added. Values for 10 μg and 30 μg of added peptide were deleted from the figure for simplicity, but showed elution traces intermediate between the curves depicted.

The ability of apoC-I and its various synthetic fragments to inhibit the interaction between rabbit apoE-enriched β-VLDL and the LRP from rat liver membranes was studied by ligand blotting (**Fig. 5**). As noted previously, apoC-I shows a concentration-dependent inhibition of the binding by apoE-enriched β-VLDL to the LRP, whereas apoC-III shows little or no inhibition even at a peptide–apoE ratio of 3:1 (18). To average our results with the various apoC-I fragments over different experiments, autoradiograms comparable to those in Fig. 5 from multiple ligand blots were subjected to scanning densitometry, and peak areas (expressed as a percentage of control values) were plotted (**Fig. 6**). Each of the apoC-I fragments, including the largest peptide, showed little or no inhibition of binding even at the highest peptide–apoE ratio (3:1, w/w). Equivalent ligand binding results were obtained when the β-VLDL used was enriched at either a 1:1 or 2:1 ratio of apoE to β-VLDL protein. The results were similar whether the samples used for ligand blotting were incubated just before the probing procedure or were obtained from the identical incubations used for studies of apoE displacement and were stored at 4°C for 2 days before blotting.

To investigate whether the amino-terminus of apoC-I contributed to these functions, the amino-terminal cyanogen bromide fragment (residues 1–38, ending in homoserine) was isolated and used for studies of apoE displacement from β-VLDL and ligand blotting. As shown in Fig. 4B, the apoC-I_{1-38} peptide was ineffective at displacing apoE from β-VLDL and was most nearly comparable to the shortest synthetic peptide, C-I_{40-57}. In ligand blotting (Figs. 5 and 6), the apoC-I_{1-38} peptide was a poor inhibitor of β-VLDL binding to the LRP.

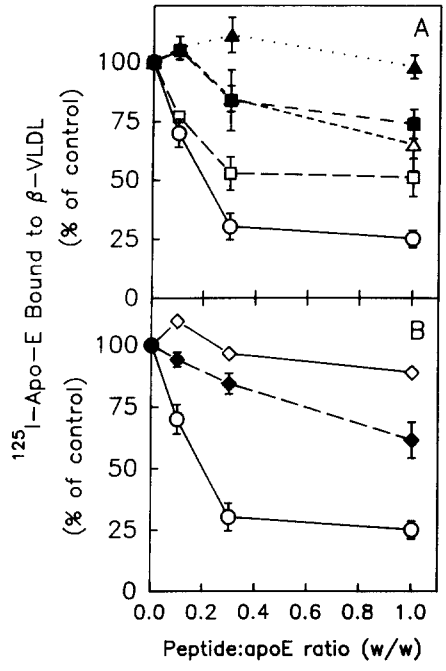

Fig. 4. Ability of peptides to displace ^{125}I-labeled apolipoprotein E from β-VLDL. Incubations and FPLC gel filtration were performed as described in the legend to Fig. 3. The amount of radiolabel contained in the β-VLDL fractions (tubes 15–18) was summed, normalized against the control incubation, and plotted versus the amount of peptide added. Panel A, apoC-I (○); C-I_{10-57} (□); C-I_{20-57} (■); C-I_{30-57} (△); C-I_{40-57} (▲). Panel B, apoC-I (○); apoC-III-1 (◆); C-I_{1-38} (◇). The points represent the mean of three experiments (± SD) for peptide:apoE weight ratios of 1:1, and of two experiments (± range) for the remaining ratios, except for C-I_{1-38}, for which only one set of data was obtained.

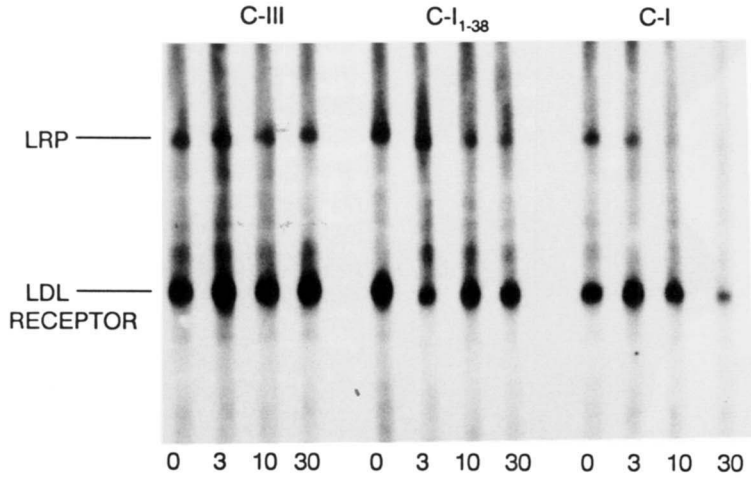

Fig. 5. Ligand blotting of apolipoprotein E-enriched β-VLDL to partially purified low density lipoprotein receptor-related protein and low density lipoprotein receptors. PVDF membrane strips containing a DEAE-cellulose fraction of rat liver membranes containing both LRP and LDL receptors were prepared as described (16). Replicate strips were blotted in the presence of biotinylated β-VLDL (10 μg protein/ml), which had been preincubated with rabbit apoE (10 μg/ml) and the indicated amount (in μg) of apoC-I peptide fragment. Bound β-VLDL were detected by incubation with ^{125}I-labeled streptavidin (1.5 × 10^6 dpm/ml; 10,800 dpm/ng), after which the strips were placed on XM film (3M Co.) for 3 h at −20°C with an intensifying screen. The strips were calibrated with the following molecular weight markers: apoB-100 (512 kDa), myosin (200 kDa), phosphorylase (97.4 kDa), and bovine serum albumin (69 kDa).

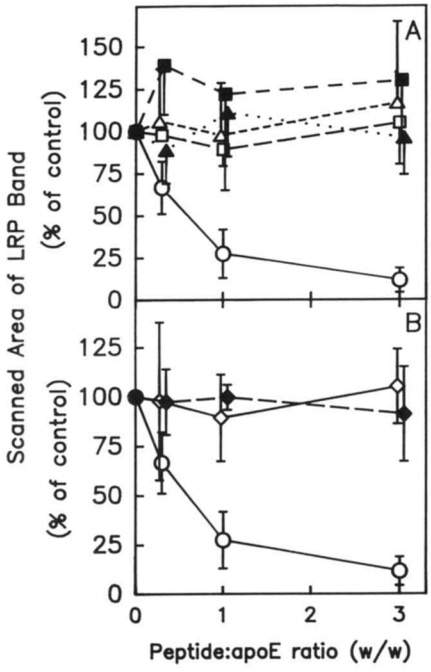

Fig. 6. Inhibition of the binding of apoE-enriched β-VLDL to partially purified LRP and LDL receptors by apoC peptides and peptide fragments. Ligand blotting was performed as described in Fig. 5 and autoradiograms from three to four experiments for the C-I peptides (two for the C-III data) were subjected to densitometric scanning to determine the area under the LRP zone. The figure depicts the amount of apoE-enriched β-VLDL bound to the LRP as a percentage of the control sample (no peptide added) in each experiment. Panel A, apoC-I (○); C-I_{10-57} (□); C-I_{20-57} (■); C-I_{30-57} (△); C-I_{40-57} (▲). Panel B, apoC-I (○); apoC-III-1 (♦); C-I_{1-38} (◇).

To assess whether the ability of the various peptides to displace apoE from the β-VLDL might correlate with affinity for a lipid surface, measurements were made of the binding of the various C peptides for vesicles prepared from DMPC. In one series of experiments, a solution containing the peptide of interest was "titrated" by the addition of unilamellar vesicles of DMPC, and the change in the tryptophan emission maximum was assessed (**Fig. 7**); the single tryptophan in apoC-I is at position 41 and thus is found in all of the peptides. Among the synthetic fragments only C-I_{10-57} was affected by DMPC to the same extent as intact apoC-I, while peptides C-I_{40-57} and C-I_{30-57} showed no effect of phospholipid on the tryptophan environment. Kinetic studies of the interaction between the peptides and multilamellar vesicles yielded similar results (data not shown). Specifically, the rate of clearing of phospholipid turbidity was rapid for apoC-I and the C-I_{10-57} peptide; the C-I_{20-57} and C-I_{30-57} peptides reacted much more slowly, and the C-I_{40-57} showed almost no clearing of liposome turbidity over the 30-min period during which measurements were taken. Very slow or no clearing of phospholipid turbidity was observed with C-I_{1-38} and C-I_{39-57}, as well as with a 1:1 (w/w) mixture of the two peptides. Similar conclusions were obtained by CD studies of peptides with and without added DMPC (**Table 2**). Addition of DMPC to the C-I_{10-57} peptide resulted in formation of a complex with almost as much α-helix as the native apoC-I, but adding phospholipid to the longer peptides produced only a low percentage of helix and/or little change in secondary structure. The

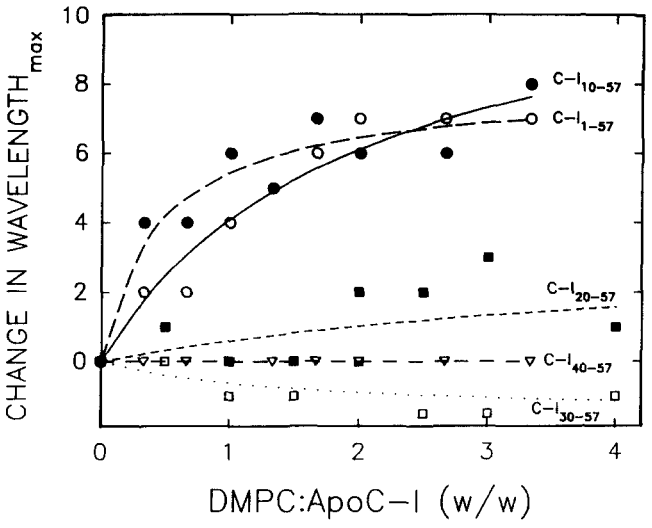

Fig. 7. Titration of lipid affinities of apolipoprotein C-I peptide fragments by monitoring fluorescence blue shifts of the intrinsic tryptophan fluorescence. The fluorescence emission spectrum of apoC-I peptide fragments was obtained 5–10 min after the addition of aliquots of a DMPC unilameller vesicle preparation; the change in maximum wavelength relative to the initial spectrum is plotted versus the weight ratio of DMPC added to the peptide.

intermediate-size peptide (C-I$_{20-57}$) showed a small effect of DMPC on helix content, as well as a small effect of the lipid on tryptophan fluorescence (Fig. 7).

DISCUSSION

The apolipoproteins have attracted considerable interest because they not only constitute structural components of the plasma lipoproteins, but also play key roles in the metabolism of lipoproteins. Direct roles include recognition of cellular receptors and modulation of enzymatic activities. Evidence is also accumulating for indirect roles, such as the effect of C apolipoproteins at blocking the clearance of triglyceride-rich particles, such as the chylomicrons, from the plasma. The biochemical basis for such actions remains to be elucidated. The present studies were directed at clarifying the mode of action of apoC-I in modulating the binding of β-VLDL to hepatic membranes. Previous workers have already established that the carboxyl-terminal 41 residues of apoC-I possess 100% of the ability of intact apoC-I to activate the LCAT enzyme (27). This finding suggests that functional properties of apoC-I might be localized to regions of primary structure, similar to the receptor-recognition sequences reported for the apoE and apoA-I (10, 28).

Our studies were designed to investigate whether partial sequences from apoC-I might be as competent as the intact form in displacing apoE from β-VLDL or inhibiting its binding to the LRP. However, unlike the LCAT activation studies, ours showed that only the intact apoC-I possessed significant ability to block interaction with LRP. That is, fragments including only the amino- or the carboxyl-terminus of apoC-I had little or no potency in blocking binding of apoE-enriched β-VLDL to either the LRP or the LDL receptor on membrane blots (Figs. 5 and 6). This suggests that either the entire apoC-I molecule is necessary to maintain a functional conformation, or the functional portions of the apoC-I are discontinuous and require sequences from both the carboxyl- and amino-termini. It can be noted, however, that intact C-I appears to inhibit the interaction of apoE-enriched β-VLDL with both the LRP and the LDL receptor (Fig. 5 and ref. 18).

Studies of the displacement of apoE from apoE-enriched β-VLDL suggest that although the longer peptides, in general, were more effective than shorter peptides, even a fragment corresponding to 84% of the native protein could not equal the potency of apoC-I in this regard (Figs. 3 and 4). None of the apoC-I fragments at the concentrations used in these studies were capable of displacing more than 50% of the labeled apoE from the β-VLDL, while intact apoC-I displaced 75–80% of the apoE. Retention of about 50% of the exogenously added apoE on β-VLDL has previously been suggested to be sufficient to mediate binding to the LRP (18). Thus, the failure of the various peptides to inhibit binding to the LRP may correlate with inability to displace more than 50% of the exogenous apoE, supporting the hypothesis that apoC-I influences binding primarily or exclusively through apoE displacement on this particular class of lipoproteins. The alternative hypothesis, as proposed by Brown and Goldstein (21) and others (22, 29), is that apoC-I (or other C apolipoproteins) binds directly to apoE, altering its conformation to one lacking the ability to recognize LRP. Our data do not disprove this hypothesis but do imply that the entire C-I molecule would be re-

TABLE 2. Calculated secondary structure of lipid-free peptides and peptide:DMPC complexes

	% α-Helix[a]	
Peptide	Peptide Alone	Peptide:DMPC Complex
C-I$_{1-57}$	32	53
C-I$_{10-57}$	18	42
C-I$_{20-57}$	9	15
C-I$_{30-57}$	5	9
C-I$_{40-57}$	0	2
C-I$_{1-38}$	10	12
A-I	55	72

[a]Determined from the molar ellipticities at 222 nm as described in reference (26) using the following mean residue weights: C-I$_{1-57}$, 116.3; C-I$_{10-57}$, 119.7; C-I$_{20-57}$, 120.6; C-I$_{30-57}$, 121.5; C-I$_{1-38}$, 111.3; C-III, 110.9; A-I, 115.2.

quired to bind to apoE and alter its conformation. It is plausible that the mechanism of apoC-I inhibition of binding might be dependent on the particular lipoprotein being studied.

Studies of phospholipid binding properties of the various peptide fragments of apoC-I indicated that only the largest fragment (C-I_{10-57}) came close to possessing the lipid affinity of the intact apoC-I (Fig. 7, Table 2). The markedly reduced affinity of the remaining peptides for phospholipid bilayers could readily account for their relative inability to displace apoE from β-VLDL or to inhibit binding to the LRP. However, the strong lipid affinity of the C-I_{10-57} peptide, as well as the significant displacement of apoE from β-VLDL, suggest that other properties, such as the absence of primary structure needed for specific protein–protein interactions, may be required to explain the inability of this peptide to inhibit the binding of apoE-enriched β-VLDL to the LRP.

Analysis of the helix-forming properties of the apoC-I sequence has predicted the existence of two extended regions of Class A amphipathic helix, spanning residues 7–33 and 35–54 (30). The middle of this molecule is characterized by a region of relatively low hydrophobic moment that may diminish the lipid binding affinity in this region. The portions of the molecule with the highest hydrophobic moment/residue are found toward the amino-terminal end (residues 7–17) and the carboxyl-terminal region (residues 40–50); apparently both of these regions must be present simultaneously in a fragment to duplicate the lipid binding affinity of intact apoC-I. It is possible that the absence of residues 7–9 from peptide C-I_{10-57}, which could complete the region encoded by exon 1, might account for the failure of this peptide to mimic intact apoC-I in its inhibition of β-VLDL binding to the LRP. In this regard, it is interesting to note that the cyanogen bromide peptides of apoC-I (C-I_{1-38}, C-I_{39-57}) were found to be uninvolved at activating LCAT (27). In our experience, these peptides, as well as C-I_{40-57}, had low or no ability to bind to DMPC vesicles. Similarly, our studies showed that peptides C-I_{1-38} and C-I_{40-57} had no ability to displace apoE or to inhibit interaction with the LRP. Thus, it may be that the amino-terminal region per se possesses no functional domain, but is required to yield a completely functional molecule by means of interaction with the carboxyl terminus possibly involving exon-encoded amphipathic helical regions.

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