Structural features of synthetic peptides of apolipoprotein E that bind the LDL receptor

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Abstract Apolipoprotein (apo) E, via its receptor binding domain contained in residues 140-150, mediates hepatic and peripheral tissue binding of cholesterol-rich lipoproteins. Previously, we reported that a synthetic peptide representing a linear tandem repeat of amino acids 141-155, the 141-155 dimer, binds the low density lipoprotein (LDL) receptor. To define the structural features essential for LDL receptor binding of the 141-155 dimer, a series of modified peptides were synthesized. The secondary structure content of the modified apoE peptides was assessed by circular dichroism (CD) and the receptor activity was studied in cellular LDL receptor binding assays. α-Helix content was necessary but not sufficient for receptor activity because both a 129-162 monomer and the 141-155 dimer peptides had comparable CD spectra and helix contents, but only the 141-155 dimer was receptor active. Deletion of the charged amino terminal residues including arg^{142} and lys^{143} in the 145-155 or 144-150 dimers had no effect on α-helix content, yet abolished their receptor activities. Helical net models of all receptor active peptides indicated that the LDL-receptor binding activity of the 141-155 dimer is dependent on at least two clusters of basic amino acids present on the hydrophilic face of the amphipathic α-helix of the 141-155, 141-150, 141-155 ($lys^{143}\rightarrow ala$) and 141-155 ($arg^{150}\rightarrow ala$) dimer peptides.—**Dyer, C. A., D. P. Cistola, G. C. Parry, and L. K. Curtiss.** Structural features of synthetic peptides of apolipoprotein E that bind the LDL receptor. *J. Lipid Res.* 1995. **36:** 80-88.

Supplementary key words circular dichroism • amphipathic helix • peptide structure modeling

Apolipoprotein (apo) E associated with cholesterol-rich lipoproteins mediates their uptake by the liver and peripheral tissues (1). ApoE is essential for the metabolism of very low density lipoproteins (VLDL) and intermediate density lipoproteins (IDL), as well as chylomicron and VLDL remnants. A direct inverse relationship between circulating levels of plasma apoE and cholesterol has been documented. Transgenic mice over-expressing apoE have decreased levels of cholesterol, whereas, mice deficient in apoE have elevated plasma cholesterol that is dramatically increased upon cholesterol feeding (2, 3). In a short period of time these apoE-knockout mice develop advanced atherosclerosis, a disease not found in normal mice. In humans, mutations of apoE or its complete deficiency result in greatly increased susceptibility to the development of atherosclerosis (4).

ApoE binds the LDL receptor, which has been shown to be essential for high affinity hepatic clearance of plasma cholesterol. At least two other cellular receptors bind apoE, a receptor expressed by the liver that binds apoE-enriched remnant particles, currently thought to be the LRP/α_2-macroglobulin receptor, and a VLDL receptor expressed predominately by the heart that binds apoE-containing triglyceride-rich lipoproteins (5, 6). Additional apoE receptors have been functionally defined and may or may not be related to the cloned receptors, LDL, LRP/α_2-macroglobulin and VLDL receptors (7).

ApoE is made of 299 amino acids and the specific domain involved in its binding to the LDL receptor has been localized to amino acids 140-150 (1). We previously reported that a synthetic peptide of apoE containing the tandem linear repeat of amino acids 141-155, a 141-155 dimer apoE peptide, binds the LDL receptor (8). In this study we explore in depth the structural features of apoE synthetic peptides that are required for binding to fibroblast LDL receptors.

EXPERIMENTAL PROCEDURES

Peptides

All peptides were synthesized by the solid phase method of Merrifield on an Applied Biosystems model 430A automated peptide synthesizer as described (8). The peptides were highly purified by preparative HPLC (Waters Autos 500) and their amino acid compositions

Abbreviations: VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; CD, circular dichroism; PBS, phosphate-buffered saline; TFE, trifluoroethanol.

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TABLE 1. Characterization of the apoE synthetic peptides

Peptide	Sequence	No. of Amino Acids	Purity	Amino Acid Composition Analysis (% yield)											
				A	R	B	Z	H	L	K	P	S	T	Y	V
129-162 Monomer	STEELRVRLASHLRKLRK RLLRDADDLQKRLAVYQ	34	98	100	94	106	100	110	91	103		100	100	80	95
141-155 Monomer	LRKLRKRLLRDADDL	15	94	96	101	100			101	99					
Dimer 141-155	$(LRKLRKRLLRDADDL)_2$	30	93	109	104	101			93	104					
143K-A Dimer	$(LRALRKRLLRDADDL)_2$	30	99	103	101	101			96	107					
150R-A Dimer	$(LRKLRKRLLADADDL)_2$	30	98	108	107	101			88	108					
144P-P Dimer	$(LRKPRKRLLRDADDL)_2$	30	98	104	101	98			95	104	109				
(141-150) Dimer	$(LRKLRKRLLR)_2$	20	100		105				89	113					
(145-155) Dimer	$(RKRLLRDADDL)_2$	22	93	109	99	95			102	104					
(144-150) Dimer	$(LRKRLLR)_2$	14	94		105				96	97					

were determined (**Table 1**). For use in the binding assays, the peptides were dialyzed overnight against phosphate-buffered saline (PBS), pH 7.2, before addition to the LDL binding assay.

Circular dichroism (CD)

Samples were prepared for CD as follows. Individual aliquots of peptides, in powder form, were weighed out using a Perkin-Elmer AD-4 Microbalance. The peptides were then dissolved in 0.5 ml of PBS alone or PBS containing differing quantities of trifluoroethanol. Each sample was passed through a 0.2-μm sterile filter prior to CD analysis. Spectra were accumulated on a Jasco J-600 spectropolarimeter. Initially, all samples were examined between 197 and 250 nm using a 0.1-cm path length cell. These spectra were accumulated with a 1.0-nm band width, a 0.2-nm/point step resolution, a scan speed 20 nm/min, and a time constant of 1 sec. Five scans were averaged for each spectrum. For further analysis, spectra were accumulated for selected samples down to 190 nm wavelength using a 0.01-cm path length cell. (It was not possible to accumulate data below 190 nm because of the physiological concentration of chloride ions, which absorb strongly at low wavelengths.) For these spectra, 25 accumulations were averaged and a step resolution of 1 nm/point was used. The data collected between 190 nm and 240 nm were analyzed using the program package CDPENCE by R. Boyko, D. Wishart and B. Sykes at the University of Alberta, Canada. The PROVENCHER algorithm (9) was used to calculate the approximate percentages of helix, beta, turn, and remaining structure.

LDL receptor binding

The human fibroblasts were a gift from Dr. J. Witztum of the University of California, San Diego. Fibroblasts were cultured in DMEM supplemented with 10% fetal bovine serum (Irvine Scientific, Santa Ana, CA), penicillin, and streptomycin. The cells were plated in 24-well (16 mm/well diameter) dishes at 2 × 10^5/ ml of medium and cultured for 5 days. The day before the binding assay the media were switched to DMEM containing 10% lipoprotein-depleted serum (10) to permit up-regulation of LDL receptor expression. LDL was isolated from human plasma by ultracentrifugation (11). The LDL was radiolabeled using the iodine monochloride method (10) to a specific activity of 200–800 cpm/ng and greater than 99% of the radioactivity was precipitated by 10% trichloroacetic acid. Cellular binding was assayed at 4°C as described (10). The amount of ^{125}I-labeled LDL bound in 30–60 min per culture well was normalized to the protein content of each well following protein measurements as described (8).

Modeling of peptide secondary structure

Helical wheel diagrams were constructed according to Schiffer and Edmundson (12) and helical net diagrams were constructed according to Dunnill (13). The mean helical hydrophobic moment $<\mu_H>$ and the mean hydrophobicity $<H_i>$ as described by Eisenberg, Weiss, and Terwilliger (14) were determined using the DNA Strider program (15).

RESULTS

We previously reported that a synthetic peptide, which contains a linear tandem dimer repeat of apoE residues 141–155, inhibits LDL receptor binding (8). A monomer of these same 141–155 residues does not inhibit binding. To understand the structural basis for this difference in binding activity, the secondary structures of the monomer and dimer apoE peptides were analyzed by circular dichroism (CD). CD spectra were observed under a variety of conditions to compare 141–155 monomer and dimer peptides and to determine the effects of concentration, temperature, pH, and solvent composition on their solution conformations. Spectra obtained at pH 7.2 and 23°C in PBS for the 141–155 monomer and 141–155 dimer peptide, $(141–155)_2$, are shown in **Fig. 1.** The 141–155 monomer exhibited a spectrum similar to that of a disordered peptide, with a single minimum occurring at < 200 nm (16). In contrast, the spectrum for the 141–155 dimer

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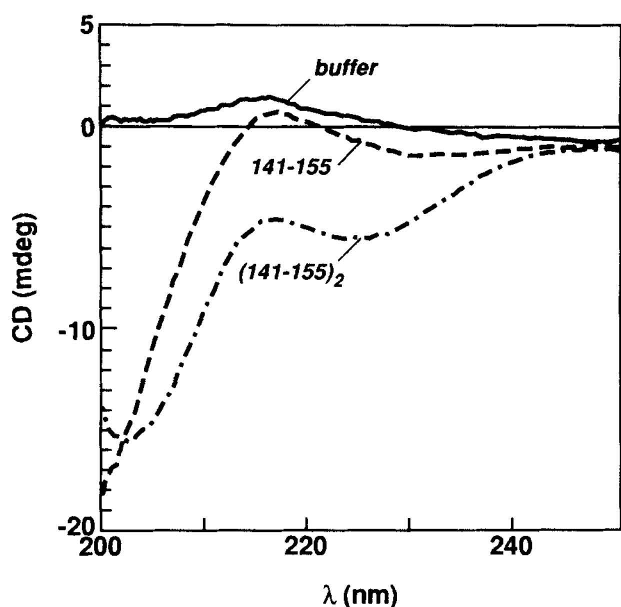

Fig. 1. CD spectra at 23°C of PBS buffer, 141-155 monomer (30.0 μM), and 141-155 dimer (30.0 μM) peptides in PBS, at pH 7.2.

exhibited a primary minimum between 200 and 210 nm, a secondary minimum around 222 nm, and a maximum near 190 nm (not shown). These features are characteristic of peptides that contain a high degree of α-helix content. Analysis of the data collected to 190 nm indicated that the dimer peptide in PBS contained approximately 70% helix, 0% beta, 0% turn, and 30% remaining (disordered) structure.

To assess the possibility of self-association of the receptor-active 141-155 dimer peptide in PBS, CD spectra were accumulated at different temperatures and sample concentrations. Spectra collected at 2, 9, 16, 30, and 37°C were essentially identical and superimposable with that shown in Fig. 1 (23°C). Also, spectra accumulated at 0.076, 0.30, 0.75, and 3.0 mg/ml were very similar. There was no trend with concentration and small quantitative differences appeared to reflect experimental scatter. Thus, there was no evidence of aggregation of the receptor-active 141-155 dimer peptide in PBS over the temperature and concentration ranges tested.

To further assess the propensity for forming α-helical structure of the 141-155 dimer peptide, CD spectra were collected for samples containing increasing amounts of trifluoroethanol (TFE) in the buffer, as shown in **Fig. 2.** TFE enhances the stability of helical peptides in solution, and this enhancement has been used as a measure of helical propensity for peptide segments that are part of larger proteins in the absence of TFE (17). As shown in Fig. 2, the CD spectra changed dramatically between 0 and 12 mol% TFE, indicating increasing α-helical structure with increasing TFE. The estimated helix content for the 141-155 dimer with 15 mol% TFE was 100%.

These observations indicated that one key structural requirement for LDL receptor binding activity of the 141-155 dimer peptide may be its α-helical conformation. The inactivity of the 141-155 monomer may reflect its inability to form a stable α-helix in solution because it contains only 15 residues. We therefore synthesized a 34-residue monomer peptide representing residues 129-162 from the native apoE sequence. We previously demonstrated that the 129-162 monomer peptide had no LDL receptor binding activity (8). A repeat examination of the activity confirmed these results. The 141-155 dimer inhibited binding of ^{125}I-labeled LDL to the fibroblast LDL receptor, whereas the 129-162 monomer was inactive (**Fig. 3A**). Averaged from five assays, the concentration at which the 141-155 dimer inhibited 50% binding was 6.5 ± 2.3 μM. When the peptide content of the synthetic peptide preparation was taken into consideration, the average effective concentration was approximately half the effective concentration previously reported (8). Interestingly, as we predicted, the CD spectra for the 141-155 dimer and 129-162 monomer peptides were comparable (Fig. 3B) and demonstrated significant α-helix. In PBS alone, the 141-155 dimer and 129-162 monomer peptides contained approximately 70% helix and in PBS with 15 mol% TFE, each contained approximately 100% helix. Therefore, although α-helical structure appeared to be required for LDL receptor binding, it alone was not sufficient for binding. These results suggested that other properties of the peptide α-helix were required for functional activity.

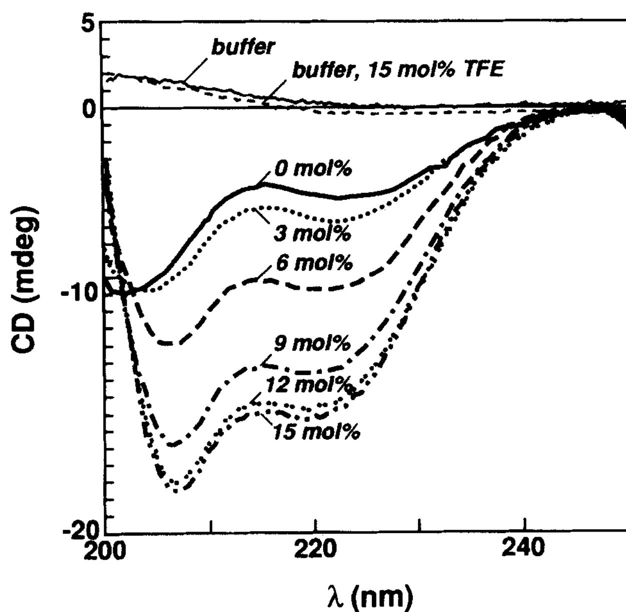

Fig. 2. CD spectra for the 141-155 dimer peptide in the presence of increasing amounts of TFE in the sample buffer. Sample conditions: peptide concentration, 30.0 μM; temperature, 23°C; pH 7.2.

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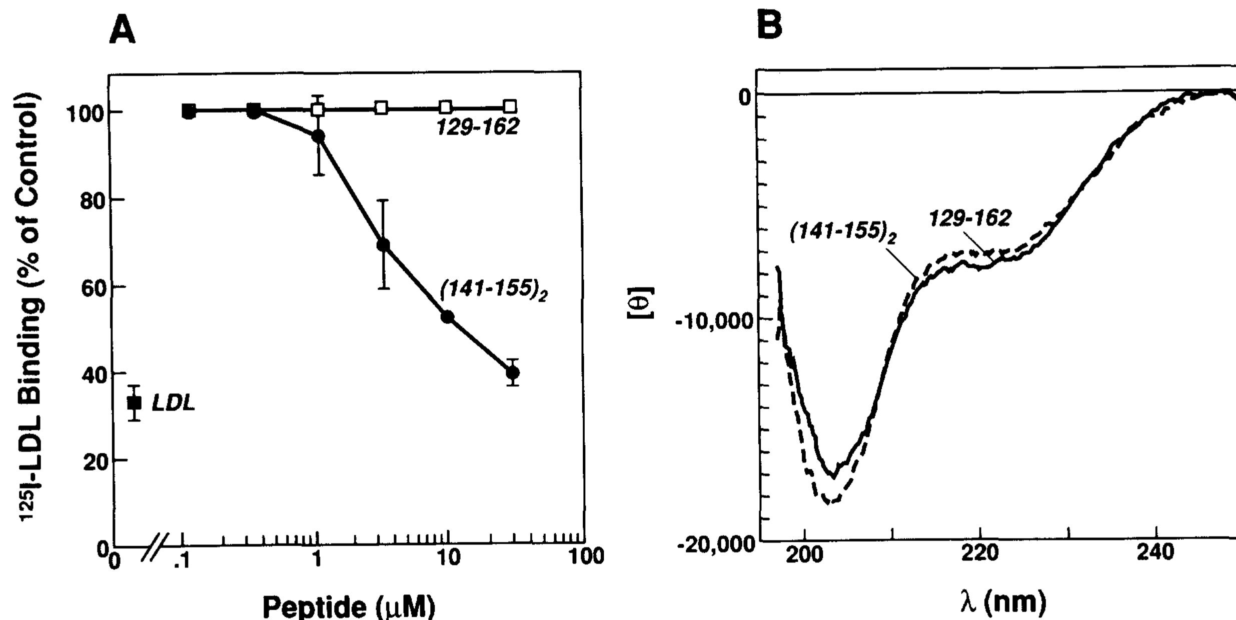

Fig. 3. LDL receptor binding activity and CD spectra analyses of the 129-162 monomer peptide and the 141-155 dimer peptide. (A) The peptides: 129-162 monomer (□) and 141-155 dimer (●) were incubated for 1 h at 4°C with human fibroblasts in the presence of 5 μg/ml (9.1 nm) of ^{125}I-labeled LDL. Control cultures bound 14.3 ± 1.9 μg of ^{125}I-labeled LDL/mg of cell protein and this was reduced to 4.8 ± 0.6 μg of ^{125}I-labeled LDL/mg of cell protein in the presence of 400 μg/ml (728 nM) of unlabeled LDL (■). Each point is the average of three replicates per treatment ± SEM. (B) CD spectra of the 129-162 monomer at 26.5 μM and the 141-155 dimer at 30 μM were measured at 23°C in PBS, pH 7.4. The abscissa represents mean residue molar ellipticity.

Verification was obtained by comparing the receptor binding activity and CD spectra of single amino acid substituted 141-155 dimer peptides. We reported earlier that the substitution of a proline for leu[144] in both repeats of the 141-155 dimer peptide abolished its receptor binding activity, whereas the substitutions of an alanine for lys[143] or arg[150] had a lesser effect on LDL receptor binding. These results are repeated in **Fig. 4A** to allow for the com-

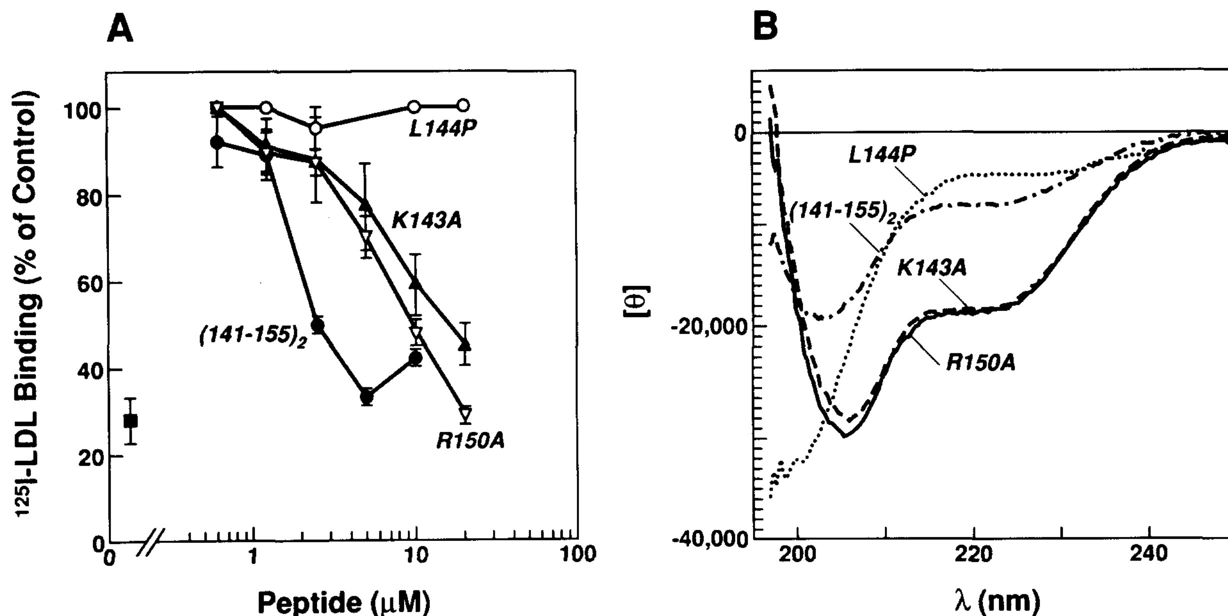

Fig. 4. LDL receptor binding activity and CD analyses of single amino acid-substituted 141-155 dimer peptides. (A) The peptides: 141-155 dimer (●), 143K→A dimer (▲), 144 L→P (♦), and 150 R→A (▼), were incubated for 1 h at 4°C with fibroblasts in the presence of 5 μg/ml (9.1 nm) of ^{125}I-labeled LDL. Control cultures bound 4.7 ± 0.5 μg of ^{125}I-labeled LDL/mg of cell protein and this reduced to 1.3 ± 0.2 of ^{125}I-labeled LDL/mg of cell protein in the presence of 400 μg/ml (728 nM) of unlabeled LDL (■). Each point is the average of three replicates per treatment ± SEM. (B) CD spectra of the same peptides (30.0 μM) at 23°C in PBS, pH 7.4. The abscissa represents mean residue molar ellipticity.

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parison to the concomitant CD analyses. As expected, the proline substitution abolished the α-helical structure of the dimer peptide; the helix content for pro for leu^{144} in PBS was 43% compared with 73% for the 141–155 dimer. In contrast, the other amino acid substitutions either maintained or slightly increased the α-helix content (Fig. 4B). These data verify that maintenance of the α-helical conformation of the dimer peptide was an essential feature for expression of LDL receptor binding activity.

To test the contribution of the amino-terminal arginine and lysine residues, we synthesized a dimer of residues 145–155 that deleted the four amino-terminal residues. To test the contribution of the carboxy-terminal aspartic acid residues, we synthesized a dimer of residues 141–150 that deleted the five carboxy-terminal amino acids. A final dimer peptide was synthesized with both the amino- and carboxy-terminal amino acid residues deleted, a 144–150 dimer peptide. Preservation of the amino terminal arginines and lysines preserved both functional activity and α-helical structure, because the 141–150 dimer peptide was equal in receptor binding activity to the 141–155 dimer peptide (**Fig. 5A, B**). In contrast, deletion of the amino-terminal residues in either the 145–155 dimer or the 144–150 dimer abolished their receptor binding activities, even though both of these peptides had comparable α-helix structure to the active 141–150 dimer. These results indicated that in addition to α-helix, the first three amino-terminal residues (leu-arg-lys) were essential for receptor binding activity; whereas the carboxy-terminal aspartic acid residues were not and may interfere with binding because the 141–150 domain was more active than the 141–155 domain.

There are numerous algorithms and models used to predict the secondary structure of apolipoproteins (18, 19). Models based on the assumption of α-helical structure were applied to all peptides that exhibited a CD spectra that was characteristic of α-helical structure. The 141–155 monomer and the proline-substituted dimer were not included because they did not exhibit a high percentage of α-helix content by CD analysis. Helical wheel diagrams of both the active and inactive peptides did not indicate a structurally unique motif (data not shown). All the peptides exhibited amphipathic character, with a positively charged face comprised of lysine and arginine residues and a hydrophobic face, enriched in leucine residues (data not shown). The CD spectra analyses of each of these peptides indicated that they had significant α-helical content (70–100%) (Figs. 3B, 4B, 5B) and the helical wheel diagrams indicated that they could assume an amphipathic α-helix that was supported by mean hydrophobic moment $<\mu_H>$ calculations. Each peptide had moderate to high $<\mu_H>$ (0.2–0.6) and very low hydrophobicities H_i (0 to −0.3).

The high mean hydrophobic moment indicated that the helical peptides were amphipathic in nature. We hypothesized that another structural feature important for activity was not just the existence of positively charged residues, but the pattern of positive charge distribution on the polar face of the amphipathic alpha helix. To examine this hypothesis we used a helical net diagram to model the

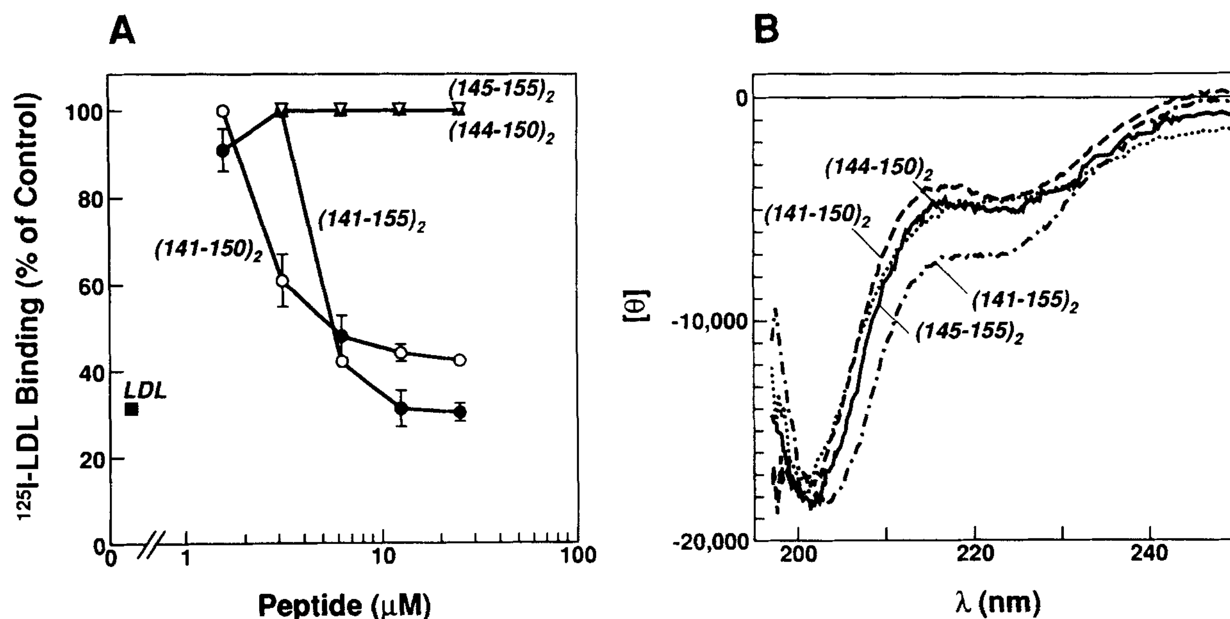

Fig. 5. LDL receptor binding and CD analyses of amino- and carboxy-terminal deleted dimer peptides. (A) The peptides: 141–155 dimer (●), 145–155 dimer (△), 141–150 dimer (○), and 144–150 dimer (▽) were incubated for 1 h at 4°C with fibroblasts in the presence of 5 μg/ml (9.1 nm) of ^{125}I-labeled LDL. Control cultures bound 5.9 ± 0.6 μg of ^{125}I-labeled LDL/mg of cell protein and this was reduced to 1.8 ± 0.1 μg of ^{125}I-labeled LDL/mg of cell protein in the presence of 400 μg/ml (728 nM) of unlabeled LDL (■). Each point is the average of three replicates per treatment ± SEM. (B) CD spectra of the dimer peptides (30.0 μM) was measured at 23°C in PBS, pH 7.4. The abscissa represents mean residue molar ellipticity.

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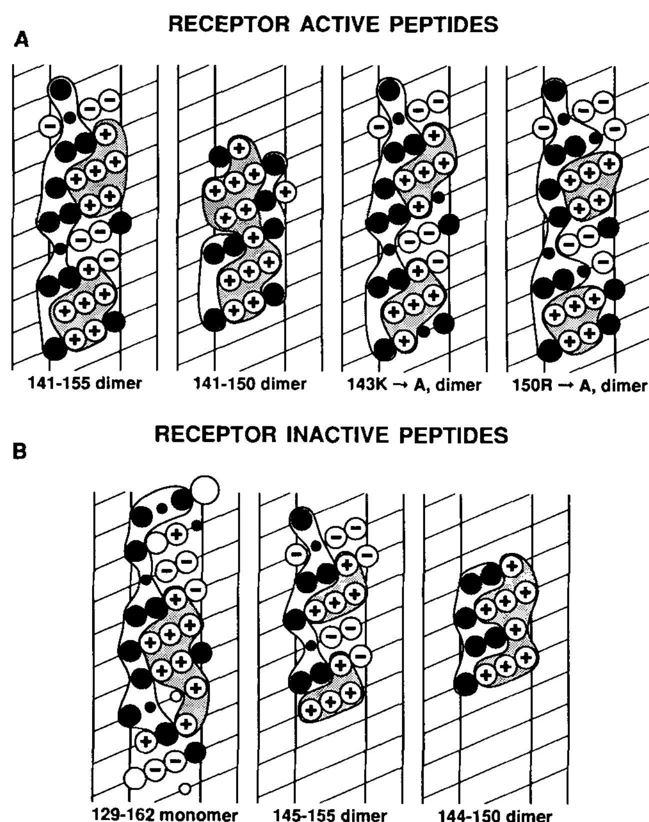

Fig. 6. Helical net diagrams of (A) receptor-active dimer peptides and (B) receptor-inactive apoE peptides. These diagrams represent the spatial grouping of the various amino acid side chains as they would appear on the face of an α-helix. The positively charged lys and arg residues (⊕), the hydrophobic residues, leu (●) and ala (●), and the negatively charged aspartic acid residues (⊖) have been outlined to highlight their clustered arrangement.

peptides (13). Each of the receptor active peptides had in common an array of two clusters of five or more basic amino acids on the polar face of the amphipathic helix (**Fig. 6A**). Both the receptor inactive 129–162 monomer and the 144–150 dimer had eight basic amino acids in only one domain on the polar face of the α-helix. The receptor inactive 145–155 dimer had two positive clusters, but each was made up of only four basic residues (Fig. 6B). All of the peptides had leucine- and alanine-rich domains clustered together along the hydrophobic face as illustrated on the α-helical net diagram (Fig. 6).

DISCUSSION

These structural studies of functional peptides of apoE that are lipid-free have enabled us to define features necessary for LDL receptor binding activity. α-Helix was necessary but not sufficient for receptor binding activity. All active peptides had significant α-helical content as determined by CD analysis. The 141–155 monomer peptide exhibited little helix content nor was it receptor-active, whereas the 141–155 dimer peptide had a high helix content and receptor activity (Figs. 1 and 3). Substituting a pro for the leu^{144} in the 141–155 dimer peptide abolished both its α-helix and receptor activity (Fig. 4). However, peptide α-helical structure alone was insufficient because the 129–162 monomer peptide and the 144–150 and 145–155 dimer peptides had α-helical content comparable to that of the 141–155 dimer peptide, yet were functionally inactive (Figs. 3, 5). The α-helical content of the synthetic apoE peptides is consistent with studies of the CD spectra of native apoE, apoE fragments (20–22), and synthetic apoE peptides (23) that contain the LDL receptor binding domain. Furthermore, the three-dimensional structure of the apoE fragment 24–166 revealed by X-ray crystallography indicates that residues 130–164 form one of the four amphipathic α-helixes (24).

The receptor binding domain of apoE is contained within amino acids 140–150 (25), and residues arg^{142}, lys^{143}, arg^{145}, lys^{146}, and arg^{150} participate in the binding of apoE to the LDL receptor (1). However, because no single substitution in native apoE results in complete loss of receptor activity, it has been proposed that binding involves multiple interactions of these lysines and arginines (26). Consistent with this proposal is our observation that single substitutions of an alanine for lys^{143} and arg^{150} in the 141–155 dimer peptide attenuated but did not abolish LDL receptor binding activity (Fig. 4A). However, the deletion of both the arg^{142} and the lys^{143} residues in the dimer 144–150 and 145–155 peptides abolished receptor binding activity (Fig. 5). Because the single substitution of lys^{143} had little effect on the activity of the 141–155 dimer (Fig. 4A), it is possible that the arg^{142} residue is more critical for LDL receptor binding. It is also possible that the loss of two basic amino acids in the amino-terminal deleted peptides was responsible for loss of receptor activity. An electrostatic potential map calculated for the 24–166 apoE fragment indicates a large region of positive potential from residues 136–150 (24). Therefore, a second structural feature for receptor binding activity appeared to be the spatial arrangement of basic amino acids on the hydrophilic face of the amphipathic α-helix that permitted clustering of a minimum charge density over a specific area. Indeed, the inactivating substitution of pro for leu^{144} occurred in the center of the cluster of basic residues required for receptor binding, disrupted the helical structure and appeared to break the positive charge density of that domain (Fig. 4). It is noteworthy that this substitution in native apoE also dramatically effects its binding to the LDL receptor (26).

The secondary structures of the exchangeable apolipoproteins such as apoA-I, A-II, A-IV, and E have been modeled with numerous algorithms (18, 19). In this study, all synthetic peptides that had significant α-helix by circular dichroic analyses were modeled using helical wheel, helical net (Fig. 6), mean helical hydrophobic mo-

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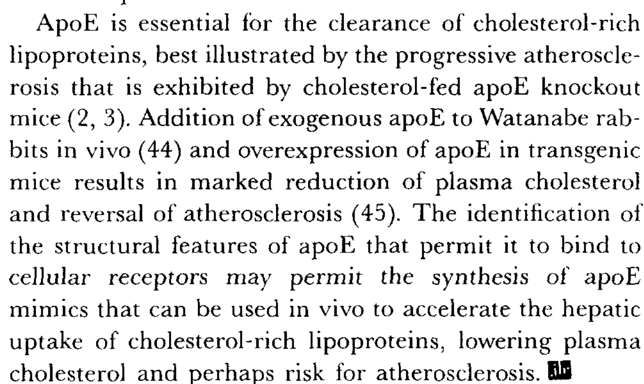
ment $<\mu_H>$, and mean hydrophobicity $<H_i>$ analyses. Helical wheel and helical net analyses indicated that all of these peptides could assume an amphipathic α-helical structure and this was consistent with published results of the amphipathic nature of residues 130-163, which form the fourth helix of the crystallized apoE fragment (24). In addition, all these peptides had moderate to high $<\mu_H>$ and very low hydrophobicity $<H_i>$, features associated with the cluster A residues 136-160 in full size apoE as reported by De Loof et al. (27). Most of these paradigms are designed to model the secondary structure of apoE when it is associated with lipid. We previously reported that a labeled 141-155 dimer peptide binds directly to cells. However, we also observed association with lipoproteins including LDL (8). Therefore, it is not known whether the LDL-associated dimer apoE peptide or the free apoE peptide in solution inhibits LDL receptor binding. Consequently, it is not clear which of the existing models is most appropriate to predict the secondary structure of the E peptides. Work in progress using 2D-NMR will generate a three-dimensional structure for the 141-155 dimer apoE peptide. These NMR spectra demonstrate a network of short interatomic distances between residues i to i+3 and i to i+4, indicative of an α-helical secondary structure.

We used helical net diagrams to model apoE peptide structure because the CD analyses indicated they had α-helix in solution in the absence of lipid. Helical nets were first proposed in 1968 to depict the spatial array of amino acids on the faces of an amphipathic α-helix while taking into account the hydrophilic/hydrophobic character of the different amino acid side chains (13). Therefore, helical net diagrams provide a picture of the longitudinal arrangement of amino acids on the helix face. Based on the helical net diagrams of the inactive and active apoE peptides (Fig. 6) we propose that a structural feature for high affinity LDL receptor binding is the presence of two clusters of a minimum number of positively charged amino acids. The LDL receptor contains seven imperfect repeats of 40 residue, cysteine-rich sequences that constitute the ligand binding domain for both apoB and apoE (28). These repeats are enriched in negatively charged amino acids. Repeat 5, which is required for the binding of apoE-rich βVLDL is unique because it has three negatively charged amino acids, one more than found in the other repeats. Russell, Brown, and Goldstein (28) proposed that multiple copies of apoE on one particle can arrange themselves to make contact with the repeat sequences of the LDL receptor. We propose that the dimer apoE peptides bind the LDL receptor by making contact with more than one of the negatively charged repeat sequences. It is proposed that four apoE molecules bind one LDL receptor (29, 30). Multiplicity of apoE on lipoproteins has been reported to be important for function. The more copies of apoE per particle, the better their affinity for and metabolism by the LDL receptor and the lipoprotein receptor-related protein (LRP) (31-37). Furthermore, the disruption of apoE multiplicity by the addition of apoCs diminishes the lipoprotein binding to the remnant receptor (38-43). We propose that the dimer apoE peptides bind the LDL receptor because they display at least two appropriately spaced clusters of positively charged residues on an amphipathic α-helix that mimics the binding of more than one apoE molecule per LDL receptor. This proposal is consistent with the lipid association of native apoEs, which is required for high affinity receptor binding (1). A phospholipid vesicle provides the spatial array by which multiple copies of apoE containing single positively charged clusters can interact with one LDL receptor.



ApoE is essential for the clearance of cholesterol-rich lipoproteins, best illustrated by the progressive atherosclerosis that is exhibited by cholesterol-fed apoE knockout mice (2, 3). Addition of exogenous apoE to Watanabe rabbits in vivo (44) and overexpression of apoE in transgenic mice results in marked reduction of plasma cholesterol and reversal of atherosclerosis (45). The identification of the structural features of apoE that permit it to bind to cellular receptors may permit the synthesis of apoE mimics that can be used in vivo to accelerate the hepatic uptake of cholesterol-rich lipoproteins, lowering plasma cholesterol and perhaps risk for atherosclerosis.

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