Role of the Arg^{123}–Tyr^{166} Paired Helix of Apolipoprotein A-I in Lecithin:Cholesterol Acyltransferase Activation*

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The Arg^{123}–Tyr^{166} central and Ala^{190}–Gln^{243} carboxyterminal pairs of helices of apoA-II were substituted with the pair of helices of apoA-I, resulting in the apoA-I(Δ(Arg^{123}–Tyr^{166}), VA-II(Ser^{12}–Ala^{75})) and apoA-I(Δ(Ala^{190}–Gln^{243}), VA-II(Ser^{12}–Gln^{77})) chimeras, respectively. The structures of these chimeras in aqueous solution and in reconstituted high density lipoproteins (rHDL) and the lecithin:cholesterol acyltransferase (LCAT) activation properties of the rHDL were studied. Recombinant human apoA-I and the chimeras were expressed in Escherichia coli and purified from the periplasmic space. Binding of the apolipoproteins with palmitoyloleylophosphatidylcholine was associated with a similar shift of Trp fluorescence maxima from 337 to 332 nm, from 339 to 334 nm, and from 337 to 333 nm, respectively. All rHDL had a Stokes radius of 4.8 nm and contained 2 apolipoprotein molecules/particle. Circular dichroism measurements revealed eight α-helices per apoA-I and per chimera molecule. The catalytic efficiencies of LCAT activation were 1.5 ± 0.33 (mean ± S.D.; n = 3), 0.054 ± 0.009 (p < 0.001 versus apoA-I), and 1.3 ± 0.32 (p = not significant versus apoA-I) nmol of cholesteryl ester/μmol, respectively. The lower LCAT activity of the central domain chimera was due to a 27-fold reduced V_{max} with unaltered K_{m}. Binding of radiolabeled LCAT to rHDL of apoA-I and apoA-I(Δ(Arg^{123}–Tyr^{166}), VA-II(Ser^{12}–Ala^{75})) was very similar. In conclusion, although substitution of the Arg^{123}–Tyr^{166} central or Ala^{190}–Gln^{243} carboxyterminal pair of helices of apoA-I with the pair of helices of apoA-II yields chimeras with structure similar to that of native apoA-I, exchange of the central domain (but not the carboxyterminal domain) of apoA-I reduces the rate of LCAT activity that is independent of binding to rHDL.

ApoA-I is synthesized as a prepropeptide, cotranslationally cleaved to pro-apoA-I, and, upon secretion, processed to mature 243-amino acid apoA-I (1). It is folded into amphipathic α-helices with hydrophilic and hydrophobic surfaces (2–4), as demonstrated with complexes of phospholipids with apoA-I or model peptides (5–11). ApoA-I, when associated with phospholipids in discoidal complexes (12–14), contains eight putative amphiphatic α-helices oriented around the edge of the disc, parallel to the acyl chains of the phospholipids, with their hydrophobic surface toward the lipid core and their hydrophilic surface toward the aqueous phase. The first amino-terminal domain (residues 44–63) has the lowest helical structure probability, whereas the second α-helix (residues 69–85) is not involved in a pair (12–14). The six carboxy-terminal α-helical structures most likely form pairs of antiparallel α-helices stabilized by protein-protein interactions. A minimum length of 17–20 amino acids (five to six helical turns) appears to be required for effective phospholipid binding and LCAT activation (12–14).

The structures in apoA-I involved in phospholipid binding and/or LCAT activation remain largely unidentified. Reported differences in LCAT activity of apoA-I and deletion mutants may result from altered folding and/or organization of these molecules in rHDL rather than from deletion of a functional domain (15, 16). Therefore, in this study, the apoA-I(Δ(Arg^{123}–Tyr^{166}), VA-II(Ser^{12}–Ala^{75})), and apoA-I(Δ(Ala^{190}–Gln^{243}), VA-II(Ser^{12}–Gln^{77}))) chimeras were produced, in which the Arg^{123}–Tyr^{166} central or Ala^{190}–Gln^{243} carboxyterminal pair of α-helices of apoA-I was deleted (Δ) and substituted (V) with the pair of α-helices of apoA-II. The average structural properties in solution and in reconstituted high density lipoprotein particles of the two chimeras were found to be unaltered, but the central domain chimera had a markedly reduced LCAT activity.

EXPERIMENTAL PROCEDURES

Oligonucleotide-directed Mutagenesis and DNA Sequencing—All DNA manipulations were carried out essentially as described by Maniatis et al. (17). Oligonucleotide-directed mutagenesis was performed by the gapped-duplex method of Kramer et al. (18) using the pMalC vector system of Stanssens et al. (19). This system employs phasmid (i.e. phage/plasmid hybrid) vectors, allowing cloning, site-directed mutagenesis, and sequencing using the same vector without recloning. Oligonucleotides were obtained by custom synthesis (Pharmacia, Brussels, Belgium). DNA sequences were determined using a primer walking strategy on an ALF DNA sequencer (Pharmacia, Uppsala). Template DNA was purified by alkaline hydrolysis followed by a polyethylene glycol precipitation step. The sequencing reactions were carried out using T7 DNA polymerase (Pharmacia) and a fast denaturing protocol as described (20). The reaction products were sized on 6% Hydrolink Long Ranger gels (AT Biochem, Malvern, PA) containing 1 × 10^{-4} M hydroxylamine to prevent deamination reactions.

1 The abbreviations used are: LCAT, lecithin:cholesterol acyltransferase; rHDL, reconstituted high density lipoprotein(s); apoA-I(Δ(Arg^{123}–Tyr^{166}), VA-II(Ser^{12}–Ala^{75})), chimera with the Arg^{123}–Tyr^{166} segment of apoA-I substituted with the Ser^{12}–Ala^{75} segment of apoA-II; apoA-I(Δ(Ala^{190}–Gln^{243}), VA-II(Ser^{12}–Gln^{77})), chimera with the Ala^{190}–Gln^{243} segment of apoA-I substituted with the Ser^{12}–Gln^{77} segment of apoA-II; POPC, palmitoyloleylophosphatidylcholine; PAGE, polyacrylamide gel electrophoresis.

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FIG. 1. Schematic representation of the predicted amphipathic helical regions in apoA-I (A), apoA-II (B), apoA-I(A)Arg123–Tyr166), apoA-II(Ser12–Ala75)), and apoA-I(A)Ala190–Gln243)), apoA-II(Ser12–Gln77)) (D). Hatched boxes, putative α-helices of apoA-I; dotted boxes, putative α-helices of apoA-II.

Deletion mutagenesis with the 5′-deoxyoligonucleotide dCAAGCGCTGGCCACGCTGTCGCTTAAGGGCTCCACCTTCT was performed on the pMC-5-apoA-I transfection vector to delete the Arg123–Tyr166 coding sequence, resulting in the pMC-5-apoA-I(A)Arg123–Tyr166) vector. By substitution of the CCG codon for Pro with the CCC codon for Pro and of the CTG codon for CAG with the TTA codon for Leu, an additional AⅡ restriction site was created. The Ser12–Ala75) segment of apoA-II was amplified by polymerase chain reaction using the 5′-deoxyoligonucleotide dCATAAECATCTGTCGAGTACCTTTCAAGCC primer, containing the apoA-II(Ser12–Thr24) sequence, and the SpI restriction site, and the 3′-deoxyoligonucleotide dATCAAATGCATGGCAGGCTGTTGCTTCAAGC primer, containing the apoA-II(Ala75–Ala75) sequence and the NsiI restriction site. Thirty cycles were performed, consisting of 1 min of denaturation at 94 °C, 2 min of annealing at 52 °C, and 1.5 min of extension at 72 °C. The reaction product was digested with NsiI and SpI and blunt-ended using T4 polymerase, and ligated into the AⅡ restriction site of the pMC-5-apoA-I(A)Arg123–Tyr166) vector, resulting in the pMC-5-apoA-I(A)Arg123–Tyr166), apoA-II(Ser12–Ala75) vector for the expression of apoA-I(A)Arg123–Tyr166), apoA-II(Ser12–Ala75) and apoA-I(A)Arg123–Tyr166), apoA-II(Ser12–Gln77) in E. coli. The pMC-5-apoA-I(A)Arg123–Tyr166), apoA-II(Ser12–Gln77) vector for the expression of apoA-I(A)Arg123–Tyr166), apoA-II(Ser12–Gln77) in E. coli was constructed as described (23).

Apolipoproteins were expressed in the periplasmic space of E. coli WK6 host cells as described (16, 23). Standard apoA-I was isolated from normolipemic human plasma as described previously (1). The purity of apoA-I was established by SDS gel electrophoresis (24) and immuno blotting (25).

Preparation of Discoidal Apolipoprotein-POPC-Cholesterol Complexes—Complexes of the apolipoproteins with POPC (Sigma) and cholesterol, at an apolipoprotein/POPC/cholesterol ratio of 1:3:0.15 (w/w/w), were prepared using the cholate dialysis procedure (11, 26). The mixture was incubated at 43 °C for 16 h, and cholate was removed by extensive dialysis. Complexes were isolated by gel filtration on a Superdex 200 HR column equilibrated with 20 mM Tris-HCl, pH 8.1, containing 0.15 M NaCl and 0.02 mg/ml sodium azide in a Waters fast performance liquid chromatography system. One-ml fractions were collected.

The sizes of these complexes were estimated by comparison of their migration positions on native 8–25% gradient polyacrylamide gels with those of standard proteins: thyroglobulin (Stokes radius of 8.5 nm), apoferritin (6.1 nm), catalase (5.2 nm), and lactate dehydrogenase (4.1 nm).

The number of apolipoprotein molecules/rHDL particle was determined following cross-linking of apolipoproteins with bis(sulfosuccinimidyl) suberate (final concentration of 1 mM) for 6 h, as described previously (27), and separation of cross-linked proteins by SDS-PAGE on 10–15% gradient gels. The extent of oligomer formation was estimated by comparison with plasma cross-linked apoA-I (27). The levels of phospholipid and cholesterol were determined using commercial enzymatic kits (Biome´rieux for phospholipid and Boehringer Mannheim for cholesterol), and the concentrations of apoA-I were determined according to Bradford (28).

Circular Dichroism Spectrometry—Circular dichroism spectra of lipid-free apolipoproteins in solution and of apolipoproteins in discoidal apolipoprotein-POPC-cholesterol complexes were measured with a Jasco J600 spectropolarimeter (Japan Spectroscopy, Tokyo) at wavelengths between 200 and 250 nm, using 6 μM sample solutions and a 1-mm path length cuvette. Backgrounds were measured at 250 nm for 5 min, followed by measurements of the a-helical content at 222 nm for 5 min. The fraction of a-helices in the secondary structure of the apolipoproteins was estimated from the molar ellipticities at 222 nm.
Table I
Properties of discoidal apolipoprotein-POPC-cholesterol complexes

| Apolipoprotein | ApoA-I<sup>a</sup> | ApoA-I/Δ(163,-Arg<sup>165</sup>),VA-II/Ser<sup>153</sup>,Ala<sup>175</sup>) | ApoA-I/Δ(Ala<sup>175</sup>,-Gln<sup>243</sup>),VA-II/Ser<sup>153</sup>,Glu<sup>273</sup>) |
|----------------|-------------------|--------------------------------|--------------------------------|
| Strokes radius (nm)<sup>b</sup> | 4.8               | 4.8                           | 4.8                           |
| ApoA-I molecules/disc<sup>c</sup> | 2                 | 2                             | 2                             |
| α-Helical content (%)<sup>d</sup> | 74                | 75                            | 72                            |
| Measured α-helices/apoA-I molecule<sup>e</sup> | 8                 | 8                             | 8                             |
| Estimated no. α-helices<sup>f</sup> | 8                 | 8                             | 8                             |
| Fraction of phospholipid surface covered<sup>g</sup> with α-helices<sup>g</sup> | 0.77              | 0.77                          | 0.77                          |

<sup>a</sup> Values for plasma apoA-I and recombinant apoA-I were identical.
<sup>b</sup> Sizes of rHDL particles were determined by PAGE.
<sup>c</sup> The number of apolipoprotein molecules/HDL particle was determined by SDS-PAGE after cross-linking with bis(sulfosuccinimidyldi) suberate.
<sup>d</sup> The α-helical content was determined by CD scanning.
<sup>e</sup> The number of α-helices/apolipoprotein molecule was calculated from the α-helical content and from the protein length assuming a length of 16 amino acids for the amphipathic helices and 6 amino acids for the adjacent β-strands (2, 12).
<sup>f</sup> Values represent the estimated number according to the model of Brasseur et al. (12) (Fig. 1).
<sup>g</sup> The phospholipid surface was calculated from the circumference of the disc, using the measured diameter minus 3 nm, multiplied by a disc height of 3.8 nm. The area covered by an α-helix containing 16 amino acids was estimated to be 4 nm<sup>2</sup>.

FIG. 4. Determination of the number of apoA-I molecules/ rHDL particle by SDS-polyacrylamide gel scanning. rHDL particles, in which apoA-I molecules were cross-linked with bis(sulfosuccinimidyl) suberate, were subjected to electrophoresis on 10–15% gradient SDS-polyacrylamide gels. A, apoA-I; B, apoA-II/Δ(Ala<sup>175</sup>,-Gln<sup>243</sup>),VA-II/Ser<sup>153</sup>,Glu<sup>273</sup>); C, apoA-I/Δ(163,-Arg<sup>165</sup>),VA-II/Ser<sup>153</sup>,Ala<sup>175</sup>); D, protein calibration mixture containing phosphorylase b (94 kDa), albumin (67 kDa), ovalbumin (43 kDa), and trypsin inhibitor (30 kDa).

\[ (\text{mol.wt.}) = \frac{30,300}{f_H} - 2340, \text{ where } f_H \text{ is the fraction of } \alpha\text{-helical structure} \]

Fluorescence Analysis—Corrected steady-state fluorescence spectra and intensities were measured as described previously (16). Excitation was carried out at 295 nm, where the contribution of tyrosyl fluorescence to the total intensity is minimal. Fluorescence was measured with a 2-mm slit width in quartz cuvettes with optical path lengths equal to 1 and 0.4 cm and wavelength resolutions of 7.2 and 3.8 nm for the emission and excitation wavelengths, respectively. Excitation was vertically polarized, and fluorescence was detected with a polarizer at a 54° angle to reduce the influence of fluorescence depolarization and brownian motion on the detected intensity. The spectra were corrected for the wavelength dependence of the emission monochromator and the photomultiplier and for background intensities as described previously (30). In the fluorescence-quenching experiments, the spectra were recorded at increasing concentrations of KI. The results were analyzed by the Stern-Volmer equation as modified by Lehrer (31):

\[ F_0/F = (1 + 1/f_KS) \times (1/KI) \]

where \( F_0 \) and \( F \) are the fluorescence intensities observed in the absence and presence of a given concentration of KI, respectively, and \( f_K \) is the fraction of quenchable fluorescence (32). The Stern-Volmer constant \( K_{SV} \) is an index of the accessibility of trypto-
**Structure-Function of ApoA-I/ApoA-II Chimeras**

**RESULTS**

Fig. 1 is a schematic representation of the predicted amphipathic helical regions in apoA-I, apoA-II, apoA-I(D(Ala(123)-Tyr(166)), VA-II(Ser(12)-Ala(75))), and apoA-II(D(Ala(190)-Gln(243)), VA-II(Ser(12)-Gln(77))). The predicted number of amphipathic helices, according to Brasseur et al. (12), was eight for apoA-I and for the two chimeras. Wild-type apoA-I and the apoA-I/apoA-II chimeras were expressed in the periplasmic space of *E. coli* cells and purified to homogeneity as evidenced by their migration as single bands on 10–15% SDS-polyacrylamide gels (Fig. 2). The molecular masses of the apolipoproteins, calculated from a plot of the logarithm of the molecular masses of the standard proteins versus the migration distance, were 28.3 kDa for apoA-I and 30.4 and 29.8 kDa for the respective chimeras and thus were in agreement with those calculated on the basis of the respective amino acid compositions. Wild-type apoA-I migrated in the same position as plasma apoA-I. The identity of each band was confirmed by immunoblot analysis using polyclonal rabbit anti-human apoA-I antibodies (data not shown).

The α-helical contents of the apolipoproteins in aqueous solution, as determined by circular dichroism scanning, were 48% for apoA-I and 43% and 41% for the respective chimeras. The recovery of apolipoproteins in discoidal apolipoprotein-POPC-cholesterol complexes after gel filtration was 90% for wild-type apoA-I and the chimeras. The Stokes radius of all rHDL was 4.8 nm (Fig. 3 and Table I). Cross-linking of the apolipoprotein molecules within all the discoidal apolipoprotein-POPC-cholesterol particles revealed 2 apolipoprotein molecules/particle (Fig. 4 and Table I). The phospholipid surface was calculated from the circumference of the disc using the measured diameter minus 3 nm, i.e. 2 × radius of an α-helix, multiplied by a disc height of 3.8 nm. The number of phospholipid molecules was calculated from the calculated surface divided by 0.45 nm², the surface area/condensed phospholipid molecule. The calculated apolipoprotein/phospholipid molar ratios were 1:90, 1:83, and 1:79 and not of the total phospholipid surface in nm² divided by 4 nm², the surface area of an α-helix, that contains 16 amino acids. The calculated fraction was 0.77 for apoA-I and the chimeras (Table I).

**TABLE II**

Fluorescence properties of apolipoproteins in rHDL particles

| Properties | ApoA-I | ApoA-I(D(Ala(123)-Tyr(166)), VA-II(Ser(12)-Ala(75))) | ApoA-II(D(Ala(190)-Gln(243)), VA-II(Ser(12)-Gln(77))) |
|------------|--------|-----------------------------------------------------|-----------------------------------------------------|
| Wavelength of maximum fluorescence (nm) | 332 | 334 | 333 |
| $K_{sv}$ (M⁻¹) for quenching of Trp fluorescence with I² | 2.9 ± 0.7 | 3.4 ± 1.1 | 1.9 ± 0.06 |
| Quenchable fraction of Trp fluorescence ($f_a$) | 0.61 ± 0.08 | 0.60 ± 0.05 | 0.58 ± 0.05 |

**DISCUSSION**

Reported differences in LCAT activity of apoA-I variants may be due to defective interaction with phospholipids, structural changes in rHDL, and/or deletion of functional domains.
To further investigate the role of the central and carboxy-terminal domains of apoA-I in LCAT activation, the apoA-I(D Arg123–Tyr166,VA-II(Ser12–Ala75)) and apoA-I(D Ala190–Gln243,VA-II(Ser12–Gln77)) chimeras were produced. The extent of in vitro phospholipid binding of these chimeras was similar to that of apoA-I, as demonstrated by comparable disc formation after mixing the apolipoproteins and phospholipids with equal weight ratios. This was evidenced by a shift of the maximum Trp fluorescence to a shorter wavelength and by a decreased accessibility of the Trp residues to I. The sizes of rHDL reconstituted with apoA-I and the chimeras were identical: the respective rHDL contained 2 apolipoprotein molecules/particle, and circular dichroism scanning revealed eight α-helices per intact apoA-I molecule and per chimera molecule, in agreement with the predicted numbers according to Brasseur et al. (12). The calculated apolipoprotein/phospholipid molar ratios of the different rHDL particles were very similar. Thus, substitution of the Ala123–Tyr166 central or Ala190–Gln243 carboxy-terminal domain helices of apo-A-I with the pair of helices of apo-A-II did not affect the size and the composition of rHDL, and the conformation and helical distribution in the different apolipoproteins in these particles were very similar. Substitution of the carboxy-terminal domain of apo-A-I with the helices of apo-A-II did not reduce LCAT activity, but substitution of the central domain resulted in a 27-fold reduction of LCAT activity, suggesting that the Ala123–Tyr166 segment is critical for LCAT activation. Binding experiments revealed that the reduced LCAT activity of the apoA-II(D Arg123–Tyr166,VA-II(Ser12–Ala75)) chimera was not due to reduced binding of LCAT to rHDL.

Based on data obtained with synthetic peptides, it was concluded that the LCAT-activating domain of apo-A-I resides in a 22-mer tandem repeat located between residues 66 and 121 (35). This was further supported by the finding that monoclonal antibodies directed against an epitope that spanned residues 95–121 inhibited the LCAT activation with apoA-I (36). Binding of antibodies to an epitope in the amino-terminal domain of apoA-I may, however, induce conformational changes in the central domain of apoA-I that may be responsible for the reduction of LCAT activity (37). Deletion of the Leu44–Leu126 amino-terminal domain of apoA-I indeed did not reduce its LCAT activity (16), suggesting that the amino-terminal domain of apoA-I is not critical for LCAT activation.

Using deletion mutants of apoA-I, Minnich et al. (38) found that deletion of the Met148–Gly186 segment resulted in decreased LCAT activity, whereas Sorci-Thomas et al. (39) found that deletion of the Pro143–Ala164 segment reduced LCAT activity. In previous studies, the conformation of deletion mutants in their respective rHDL was not investigated. However, it has been demonstrated that the decreased LCAT activity of deletion mutants of apoA-I may be due to differences in folding (decreased α-helical content) and/or organization of the apolipoproteins in rHDL (3 or 4 molecules/particle as compared with 2 for intact apoA-I) rather than to the removal of specific domains for LCAT activity (16). Indeed, it has been demonstrated that rHDL discs containing wild-type apoA-I may have discrete sizes, compositions (with 2, 3, or 4 protein molecules/particle), and apo-A-I conformations (with six, seven, or eight α-helices/apoA-I molecule in contact with lipid) and that differences in the apoA-I structure in these particles correlate with their ability to activate LCAT (40).

Chimeras in which α-helical segments of apoA-I are substituted with helical segments of apoA-II, which does not activate LCAT, in such a way that the average secondary structure of the apolipoprotein molecule as well as the organization of the apolipoprotein molecules in rHDL are not affected may be preferable reagents to address the function of a particular structural domain in LCAT activation. Indeed, rHDL containing apoA-II have a very low LCAT activity (41), and the addition of apo-A-II together with apoA-I to liposomes reduces LCAT activity by 70% (42). Thus, substitution of sequences in apoA-I that are critical for the interaction with LCAT with sequences derived from apoA-II would result in decreased LCAT activity. Substitution of the carboxy-terminal domain of apoA-I with helices of apoA-II was found not to affect LCAT activity, but substitution of the central domain resulted in a 25-fold reduction of LCAT activity, suggesting that the central domain (but not the carboxy-terminal domain) is essential for LCAT activation.

The differences in LCAT activity were due to differences in apparent $V_{\text{max}}$ values, which reflect the activated enzyme concentration, and not to differences in apparent $K_m$ values, which reflect the affinity of LCAT for rHDL (43). Similar $K_m$ values are indeed in agreement with similar binding of LCAT to rHDL of apoA-I and the central domain chimera. Thus, the Ala123–Tyr166 segment is critical for LCAT activation.

### Table III

| Apolipoprotein | Apparent $V_{\text{max}}$ (nmol CE/h) | Apparent $K_m$ (μM) | Apparent $V_{\text{max}}$/Apparent $K_m$ (nmol CE/h/μM) |
|---------------|---------------------------------|----------------|--------------------------------|
| ApoA-I        | 27 ± 3.8                        | 18 ± 3.1       | 1.5 ± 0.33                       |
| ApoA-I(D Arg123–Tyr166,VA-II(Ser12–Ala75)) | 1.4 ± 0.21                        | 25 ± 5.0       | 0.054 ± 0.009                   |
| ApoA-I(D Ala190–Gln243,VA-II(Ser12–Gln77)) | 21 ± 3.8                        | 16 ± 2.4       | 1.3 ± 0.32                      |

$^a$ CE, cholesteryl ester.

$^b$ $p < 0.001$. 

**Fig. 6.** Binding of radiolabeled LCAT to discoidal apolipoprotein-POPC-cholesterol complexes. •, apoA-I; ■, apoA-I(D Arg123–Tyr166,VA-II(Ser12–Ala75)).

**TABLE III**

| Apolipoprotein | Apparent $V_{\text{max}}$ (nmol CE/h) | Apparent $K_m$ (μM) | Apparent $V_{\text{max}}$/Apparent $K_m$ (nmol CE/h/μM) |
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Tyr^{166} segment of apoA-I appears to contain structures that are required for optimal phospholipid and cholesterol presentation to LCAT (44) that cannot be mimicked by the apoA-II segment. It is possible that substitution of the central domain affects the conformation of a hinged domain that is crucial for LCAT activation because antibodies that interfere with the mobility of a hinged domain in the central part of apoA-I inhibit LCAT activation (37). Previous data obtained with deletion mutants supported the existence of such a hinged domain overlapping the Ala^{124}–His^{162} segment of apoA-I (16). Because conformational changes elsewhere in the molecule could not be excluded, these data were, however, not conclusive. The present study strongly suggests that this hinged domain most likely overlaps the Ala^{124}–His^{162} domain of apoA-I.

In conclusion, substitution of the central or carboxyl-terminal pair of helices of apoA-I with the helices of apoA-II does not affect its average structure in rHDL. Substitution of the central domain affects the binding of LCAT to rHDL is not reduced.

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REFERENCES
1. Brewer, H. B., Jr., Fairwell, T., LaRue, A., Ronan, R., House, A., and Bronzert, T. J. (1978) Biochim. Biophys. Res. Commun. 80, 623–630
2. Segrest, J. P., Jackson, R. L., Morrisett, J. D., and Gotto, A. M., Jr. (1974) FEBS Lett. 38, 247–258
3. Segrest, J. P., De Loof, H., Dohman, J. G., Brouillette, C. G., and Anantharamaiah, G. M. (1990) Proteins 8, 103–117; Correction (1991) Proteins 9, 79
4. Segrest, J. P., Jones, M. K., De Loof, H., Brouillette, C. G., Venkatachalapathi, V. V., and Anantharamaiah, G. M. (1992) J. Lipid Res. 33, 141–166
5. Kroon, D. J., Kuperberg, J. P., Kaiser, E., and Kezdy, K. F. (1978) J. Am. Chem. Soc. 100, 5975–5977
6. Fukushima, D., Yokoyama, S., Kroon, D. J., Kezdy, F. J., and Kaiser, E. T. (1986) J. Biol. Chem. 261, 10651–10657
7. Sparrow, J. T., and Gotto, A. M., Jr. (1980) Ann. N. Y. Acad. Sci. 348, 187–211
8. Anantharamaiah, G. M., Jones, J. L., Brouillette, C. G., Schmidt, C. F., Chang, B. H., Hughes, T. A., Brown, A. S., and Segrest, J. P. (1986) J. Biol. Chem. 261, 10248–10255
9. Nakagawa, S. H., Lau, H. S. H., Kezdy, F. J., and Kaiser, E. T. (1985) J. Am. Chem. Soc. 107, 7087–7092
10. Srinivas, R. V., Venkatachalapathi, Y. U., Rui, Z., Owens, R. J., Gupta, K. B., Srinivas, S. K., Anantharamaiah, G. M., Segrest, J. P., and Compan, R. W. (1991) J. Cell. Biochem. 45, 224–237
11. Vanloo, B., Morrison, J., Fidge, N., Lorent, G., Lina, L., Brasseur, R., Ruysschaert, J. M., Baert, J., and Rosseneu, M. (1991) J. Lipid Res. 32, 1253–1264
12. Brasseur, R., De Meutter, J., Vanloo, B., Goormaghtigh, E., Ruysschaert, J. M., and Rosseneu, M. (1989) Biochim. Biophys. Acta 1043, 245–252
13. Wald, J. H., Goormaghtigh, E., De Meutter, J., Ruysschaert, J. M., and Jonas, A. (1990) J. Biol. Chem. 265, 20944–20950
14. Brasseur, R. (1991) J. Biol. Chem. 266, 16120–16127
15. Jonas, A., Wald, J. H., Tothill, K. L. H., Krul, E. S., and Kezdy, K. E. (1990) J. Biol. Chem. 265, 22123–22129
16. Holvoet, P., Zhao, Z., Vanloo, B., Vos, R., Deridder, E., Dhoest, A., Taveirne, J., Brouwers, E., Demarsin, E., ENGELBORGHE, Y., Rosseneu, M., Collen, D., and Brasseur, R. (1995) Biochemistry 34, 13334–13342
17. Mariati, T., Fritsch, E. F., and Rambour, J. (1982) Molecular Cloning: A Laboratory Manual, p. 200, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
18. Kramer, W., Drutsa, V., Jansen, H. W., Kramer, B., Pflugfelder, M., and Fritz, H. J. (1984) Nucleic Acids Res. 12, 9441–9456
19. Stanssens, P., Oppen, C., McKewen, Y. M., Kramer, K., Zabeau, M., and Fritz, H. J. (1989) Nucleic Acids Res. 17, 4441–4454
20. Zhou, C., Yang, Y., and Jong, A. Y. (1990) FEBS Lett. 263, 224–237
21. Holvoet, P., Zhao, Z., Vanloo, B., Vos, R., Deridder, E., Dhoest, A., Taveirne, J., Brouwers, E., Demarsin, E., ENGELBORGHE, Y., Rosseneu, M., Collen, D., and Brasseur, R. (1995) Biochemistry 34, 13334–13342
22. Kramer, W., Drutsa, V., Jansen, H. W., Kramer, B., Pflugfelder, M., and Fritz, H. J. (1984) Nucleic Acids Res. 12, 9441–9456
23. Stanssens, P., Oppen, C., McKewen, Y. M., Kramer, K., Zabeau, M., and Fritz, H. J. (1989) Nucleic Acids Res. 17, 4441–4454
24. Zhou, C., Yang, Y., and Jong, A. Y. (1990) FEBS Lett. 263, 224–237
25. Towbin, H., Staehelin, T., and Gordon, J. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4350–4354
26. Matz, C. E., and Jonas, A. (1982) J. Biol. Chem. 257, 4535–4540
27. Swaany, J. B. (1986) Methods Enzymol. 128, 613–626
28. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
29. Chen, Y. H., Yang, J. T., and Martinez, H. M. (1972) J. Biol. Chem. 247, 22123–22129
30. Jonas, A., Wald, J. H., Tothill, K. L. H., Krul, E. S., and Kezdy, K. E. (1990) J. Biol. Chem. 265, 22123–22129
31. Holvoet, P., Zhao, Z., Vanloo, B., Vos, R., Deridder, E., Dhoest, A., Taveirne, J., Brouwers, E., Demarsin, E., Engelborghe, Y., Rosseneu, M., Collen, D., and Brasseur, R. (1995) Biochemistry 34, 13334–13342