ROLE OF AMPHIPATHIC HELIXES IN HDL STRUCTURE/FUNCTION

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ABSTRACT

In a recent analysis we classified amphipathic helix domains into a minimum of seven distinct classes. Four amphipathic helix classes are found in lipid-associating proteins: apolipoproteins, certain polypeptide hormones, polypeptide venoms and antibiotics, and certain complex transmembrane proteins. Three amphipathic helix classes are involved in both intra- and intermolecular protein-protein interactions: calmodulin-regulated protein kinases, coiled-coil containing proteins that include the so-called leucine zipper, and globular helical proteins.

Three central hypothesis have been developed in our studies of the apolipoprotein class of amphipathic helixes: 1) The "Snorkel" hypothesis proposes that when the amphipathic helix is associated with phospholipid, amphipathic basic residues extend toward the polar face of the helix to insert their charged residues into the aqueous milieu: thus the entirety of the uncharged van der Waals'surface of the amphipathic helix is buried within the lipid. 2) We have formulated a hypothesis that Glutamyl residues located at positions 78 and 111 in apolipoprotein A-I on the nonpolar face of two amphipathic helical domains are critical to LCAT activation. 3) The hinged-domain hypothesis was proposed to explain the structural basis for the quantization of HDL subspecies, protein-protein interactions in HDL, and the HDL disc to sphere transformation.

INTRODUCTION

The amphipathic helix is an often encountered secondary structural motif in biologically active peptides and proteins. The amphipathic helix was first described as a unique structure/function motif involved in lipid interaction by Segrest *et al.* in 1974 (1). Amphipathic helical domains have been described for other lipid associating proteins, including polypeptide hormones such as endorphins, polypeptide venoms such as bombolitin, polypeptide antibiotics such as the meganins, and certain complex transmembrane proteins, such as bacteriorhodopsin (2). Based upon a detailed analysis of their physical-chemical and structural properties, we have grouped amphipathic helixes into seven classes (A, H, L, G, K, C, and M: Fig 1) (2).

Hypercholesterolemia, Hypocholesterolemia, Hypertriglyceridemia
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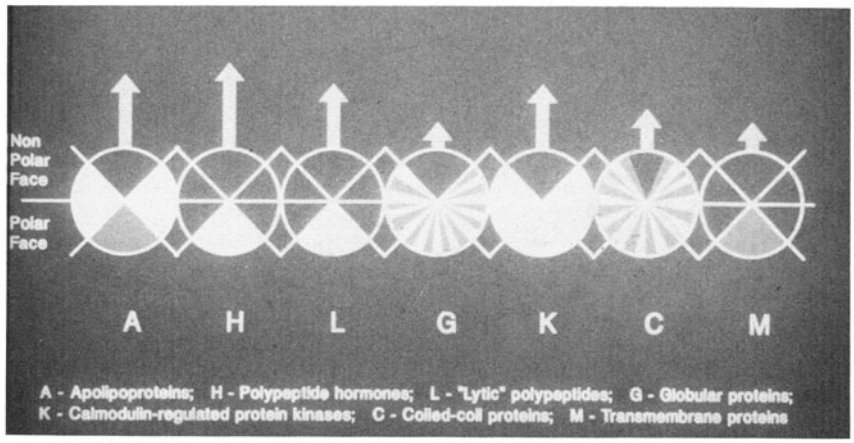


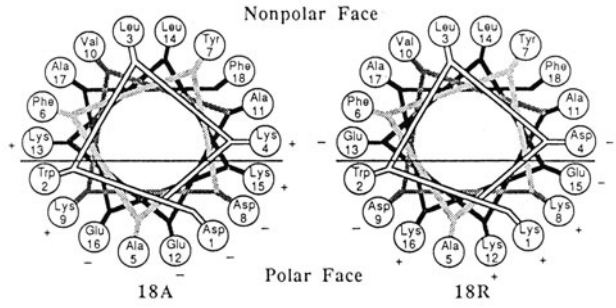
Fig. 1. Schematic representation of seven different classes of the amphipathic helix. Different circles represent each class. Arrows above each class indicate the relative mean hydrophobic moment.

The exchangeable apolipoproteins from plasma lipoproteins (apo A-I, A-II, A-IV, C-I, C-II, C-III and E) belong to the class A amphipathic helixes. They differ from the rest of the classes in that they possess positively charged residues at the polar-nonpolar interface and negatively charged residues at the center of the polar face. The most striking feature of these exchangeable apolipoproteins is the presence of internal 22-residue-long repeats (3). More importantly, this repeating unit has the periodicity of an amphipathic helix. These observations form a major basis for the investigations of the structure and functions of the apolipoproteins in our laboratory.

THE "SNORKEL HYPOTHESIS"

Our approach to understanding the factors responsible for the lipid association of the apolipoprotein class of amphipathic helix was the synthesis of model peptide analogs of this class. Our initial studies of peptide analogs involved two sets of peptides (4-7). One set of model peptides were designed to mimic the class A amphipathic helix; i.e., positively charged residues at the polar-nonpolar interface and negatively charged residues at the center of the polar face. The second set of analogs had a reverse charge distribution (Fig. 2). Examination of the lipid-associating properties of these peptide analogs showed that the class A mimicking peptide analogs interacted much more effectively to form stable discoidal peptide:lipid complexes than the analogs with the reverse charge distribution. These analogs for the class A amphipathic helix were also able to competitively displace apo A-I from HDL compared to the analogs with reverse charge distribution. From these studies we recognized that the positively charged residues at the interface, because of their longer acyl chainlength, may be playing an important role in increasing the lipid affinity of the peptide analogs of class A amphipathic helix.

As part of a study to understand the molecular properties of apo A-I we synthesized analogs for the 22mer consensus lipid-associating domain with the sequence PVLDEFREKLNE x EEALKQKLK. As can be seen in Fig. 3, the 13th position (denoted by X in the sequence) is located at the nonpolar



37aA = 18A - Ala - 18A

37pA = 18A - Pro - 18A

Fig. 2. Helical wheel representation of model 18A and 18R peptide analogs.

face, 40° from the polar-nonpolar interface of the amphipathic helix. Amino acid susbtitutions were therefore carried out in this position to see the effect of the acyl chain length on lipid affinity. Three peptide analogs were synthesized with X at the 13th position substituted by Glu, Lys and

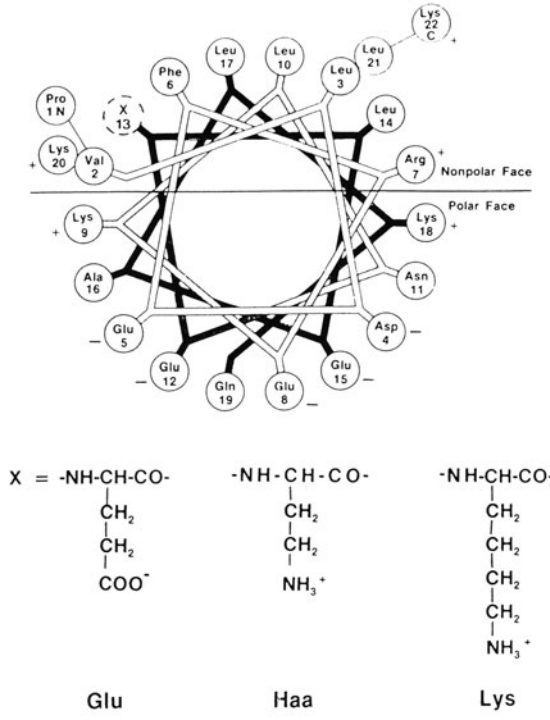


Fig. 3. Helical wheel representation viewed from the amino terminal end of the consensus 22mer peptide for the 22mer repeating units of apo A-I. X = Glu, Lys or Haa.

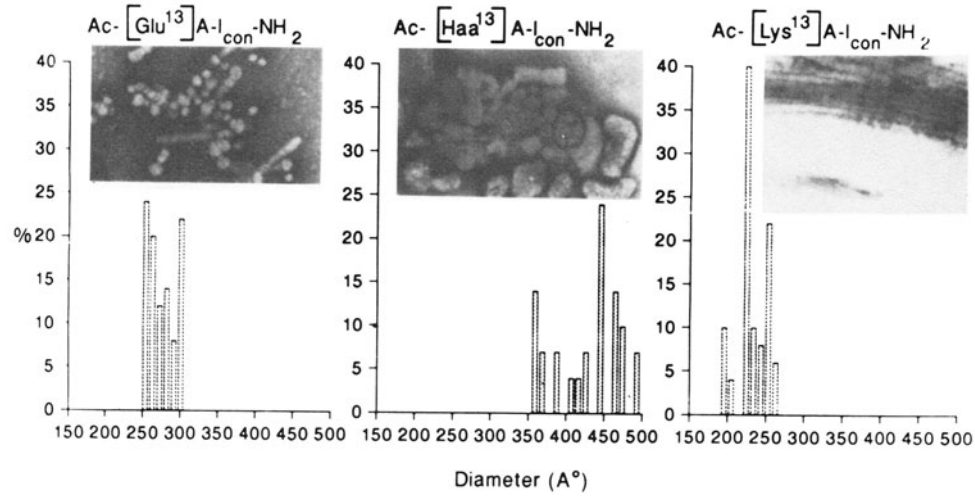


Fig. 4. (A) Negative stain electron microscopy of peptide DMPC complexes at 1:1 weight ratio. (B) Morphographic analysis of diameters of discoidal complexes.

L-homoaminoalanine (Haa). The peptide containing Haa served as a control peptide to study the effect of acyl chain length on lipid affinity because this amino acid has the same acyl chainlength as Glu but a positively charged side chain, similar to Lys. Thus the effect of the longer acyl chain length of Lys on lipid affinity when it is close to the polar-nonpolar interface was studied.

The peptides were compared for their ability to interact with multilamellar vesicles of dimyristoyl phosphatidylcholine (DMPC); the extent of interaction was estimated by their ability to clarify turbid multilamellar vesicles of DMPC and to form stable discoidal complexes. Turbidity clarification studies showed that the Lys analog was the only peptide which clarified the multilamellar vesicles of DMPC. The Lys analog formed the smallest discoidal complex of the three peptides (Stokes diameters: [Lys^{13}]A-I$_{con}$, 110 ± 30 A; [Glu^{13}]A-I$_{con}$, 200 ± 50 A; and [Haa^{13}]A-I$_{con}$, 310 ± 30 A). We have shown previously that the size of the discoidal complexes varies inversely with the lipid affinity of the peptide. Thus, based on these studies, the Lys analog has a greater lipid affinity than the other two.

Dye leakage experiments using fluorescence-entrapped egg PC liposomes also suggested that the Lys analog possesses increased lipid affinity compared to the other two analogs. The results of the fluorescence leakage are shown in Fig. 5. The results show that [Lys^{13}]A-I$_{con}$ at 200 g equals Triton X-100. The other two analogs at the same peptide concentration released only about 50% of the entrapped dye.

Based on these results we propose the "Snorkel" model shown in Fig. 6 for the lipid association of apolipoprotein class amphipathic helix. The bulk of the van der Waals surface areas of the positively charged residues are hydrophobic. We propose that the amphipathic basic residues, when associated with phospholipid, extend toward the polar face of the helix to insert their charged moieties into the aqueous milieu for solvation. We suggest that essentially all of the uncharged van der Waals surface of the amphipathic helical domains of the apolipoproteins can be buried within the interior of a phospholipid monolayer. Compared to the other classes of the amphipathic helix, the arrangement of the charged residues found in the

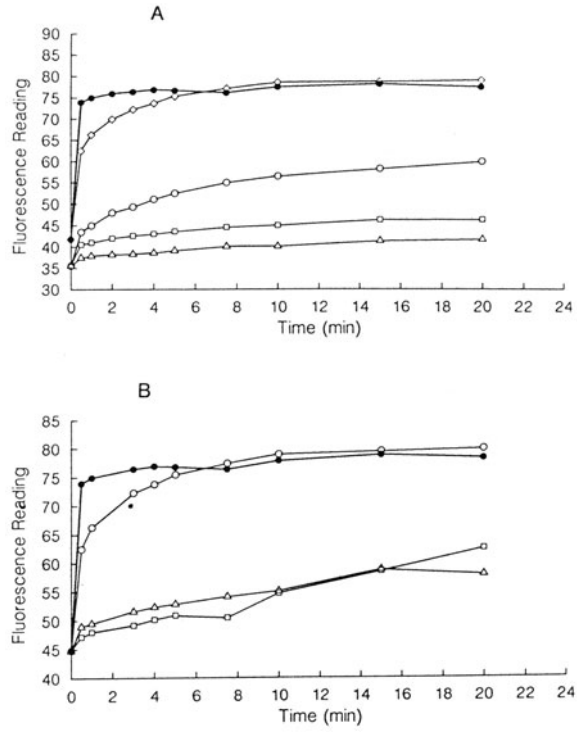


Fig. 5. Fluorescence dye leakage from egg PC entrapped vesicles: Carboxy fluorescein was trapped in small unilamellar egg PC vesicles. Perturbation by the protein of the lipid bilayer was monitored by the rate of leakage and dequenching of fluorescent dye into the media. A. Triton X-100 ● - ● and ◆ - ◆ 200 vg, ○ - ○ 100 vg, □ - □ 50 vg and ▲ - ▲ 25 vg of A-I_{con} Lys^{13}. B. ○ - ○ A-I_{con} Lys^{13}, □ - □ A-I_{con} Haa^{13} and ▲ - ▲ A-I_{con} Glu^{13}.

apolipoproteins provides for a deeper helix insertion into a monolayer and thus a greater lipid affinity.

MECHANISMS OF LCAT ACTIVATION BY APO A-I

Apolipoprotein apo A-I is the major co-factor for activation of the plasma enzyme lecithin:cholesterol acyltransferase (LCAT). LCAT is thought to mediate reverse cholesterol transport by trapping cholesterol in the form of cholesteryl ester in the HDL particle for removal by the liver. Many apolipoproteins also can serve as co-factors for the enzyme LCAT. However, the extent of activation for these proteins is similar to that of the nonspecific activators such as non-homologous synthetic peptides (5). Human apo A-I has therefore been studied extensively to localize the LCAT activating domain(s) in its sequence (6, 7). Based on studies of the CNBr fragments and the synthetic peptides corresponding to the CNBr fragmentation products the LCAT activating domain(s) could not be localized. We therefore examined the repeating 22mer units of apo A-I to attempt to understand the structure and function of apo A-I.

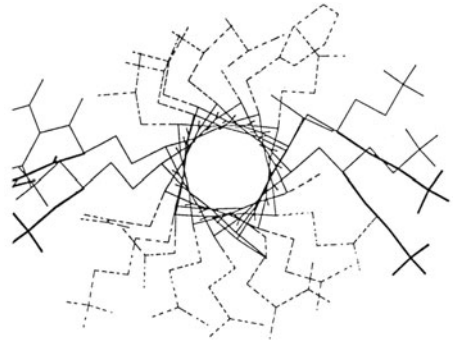


Fig. 6. Computer model of an amphipathic peptide indicating features of the "Snorkel" model. The consensus class A apolipoprotein amphipathic helical peptide (apo $A\text{-}I_{con}$) was modeled using the "SYBYL" program package (TRIPOS Associates Inc.). An idealized helix was built with backbone dihedral angles Y = 58° and F = -47°. One side chain dihedral angle of each of the four positively charged residues was changed from the trans to gouche conformation so as to bring the end of the side chains closer to the hydrophilic side of the helix. Both the initial and the "snorkel" structure were subsequently energy minimized with SYBYL using AMBER force-field parameters. A superposition of the two minimized helixes is shown in the figure. The energy difference between them was very small, showing the "snorkel"-configuration is possible without bad intramolecular steric constraints. Positively charged residues are shown in solid light (initial) and dark (snorkel).

There are eight 22mer tandem repeats at the C-terminal end of apo A-I. In every case but one, the eight tandem 22mer repeats are punctuated by a single proline. Each tandem 22mer repeat in apo A-I is an amphipathic helix. Because each tandem 22mer in apo A-I appears to represent a duplication of a primordial gene sequence (8), as part of our studies on the molecular properties of amphipathic helixes, a consensus sequence approximation of the primordial 22mer was derived. The sequence, called $A\text{-}I_{con}$, is as follows: Pro Val Leu Asp Glu Phe Arg Glu Lys Leu Asn Glu X Leu Glu Ala Leu Lys Gln Lys Leu Lys (Fig. 3). This sequence represents the most prevalent amino acid residue at each position of the eight 22mers. Although the 13th residue is positioned at the nonpolar face of the consensus sequence, six out of the eight tandem repeats of apo A-I have a charged residue at this position. The anomalous nature of this residue suggested to us the possibility that this position might play a role in the LCAT-activating properties of apo A-I. To investigate this possibility we synthesized four 22mer peptide analogs differing only in the 13th position. Three homo and one heterodimeric combinations were synthesized (Table 1).

Table 1.

E	PVLDE FREKL NEELE ALKQK LK
R	PVLDE FREKL NERLE ALKQK LK
K	PVLDE FREKL NEKLE ALKQK LK
H	PVLDE FREKL NEHLE ALKQK LK
EE	PVLDE FREKL NEELE ALKQK LK PVLDE FREKL NEELE ALKQK LK
RE	PVLDE FREKL NERLE ALKQK LK PVLDE FREKL NEELE ALKQK LK
RR	PVLDE FREKL NERLE ALKQK LK PVLDE FREKL NERLE ALKQK LK
HH	PVLDE FREKL NEHLE ALKQK LK PVLDE FREKL NEHLE ALKQK LK

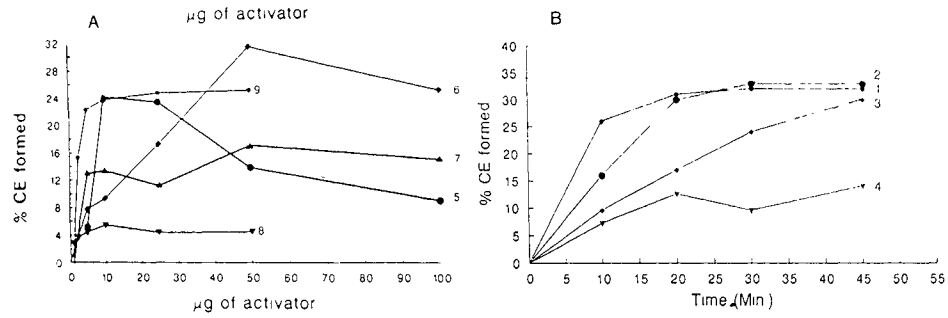


Fig. 7. Activation of LCAT by synthetic peptide analogs as measured by: (A) egg lecithin small unilamelar vesicle procedure; (B) egg lecithin discoidal particle prepared by the cholate dialysis procedure (10, 13).

These peptides were studied for their ability to activate the enzyme LCAT using two different assay systems: i) the egg PC vesicular assay, and ii) the discoidal assay method using the cholate dialysis procedure (10, 13). The results were compared with the ability of apo A-I to activate LCAT in these two assay systems. The results of the LCAT activation using the dimer peptide analogs are shown in Fig. 7. In both assay systems the homodimer analog containing Glu at the 13th position equaled apo A-I in its ability to activate LCAT.

Examination of the order of distribution of 13th amino acid in the eight tandem repeats of apo A-I (Fig. 8) shows that residues 66-121 of apo A-I are similar to the Glu-dimer analog. We therefore propose that the major LCAT activating domain of apo A-I resides in 66-121 region of apo A-I. These results thus explain the reason for failure in localizing the LCAT activating domain of apo A-I. The 66-121 region of apo A-I contains three Methionyl residues. CNBr cleavage would thus fragment this region of apo A-I and the fragments would be devoid of 66-121 apo A-I sequence. Recent studies by Jonas *et al.* (12) provide additional support for this hypothesis.

These results led us to believe that glutamic acids at residues 78 and 111 of apo A-I are critical for the LCAT activating ability of apo A-I. With a view to test this we have prepared recombinant apo A-I and a mutant apo A-I in which the 78th Glu residue is changed to Ala. These two proteins were compared with human apo A-I for their ability to activate the enzyme LCAT following the assay systems used for synthetic peptide analogs. Results from these preliminary LCAT studies are shown in Fig. 9. These results indicate that the Glu^{78} -> Ala apo A-I is half as effective in

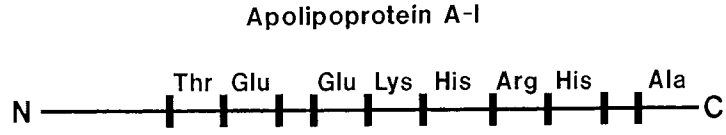


Fig. 8. Order of distribution of 13th amino acid in the eight tandem 22mer repeats of apo A-I. Two vertical lines between the amino acid residues represent 22mer. The peptide ([Glu 13]A-I_{con})2 is similar to the two glutamic acid-containing 22mers in having glutamic acid residues at the same position as the two 22mers.

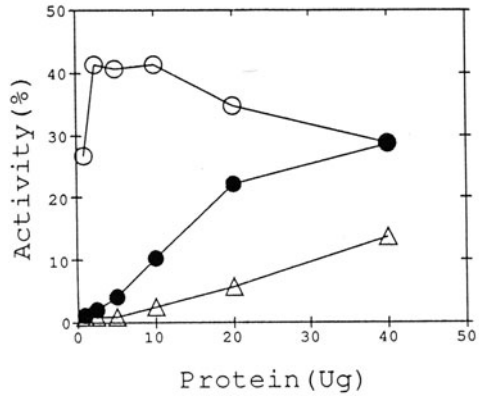


Fig. 9. Activation of LCAT by: (1) plasma apo A-I, (2) recombinant apo A-I and (3) mutant (Glu^{78} -->Ala) apo A-I as measured by the egg lecithin small unilamellar vesicles assay method.

activating the enzyme LCAT as the recombinant apo A-I with the sequence identical to that of plasma apo A-I. These results support the hypothesis that sequence specific glutamic acid residues are important for the LCAT activation by apo A-I.

THE "HINGED-DOMAIN" HYPOTHESIS

Analysis of the complexes formed between apo A-I and DMPC has shown the formation of a highly controlled stoichiometric class of particles of discrete size containing 2 apo A-I molecules (R-2 complexes) and three apo A-I molecules (R-3 complexes) (11). Variation of the molar ratio of lipid to protein produced discoidal complexes which varied in diameter by a uniformly spaced increment. These observations led to the proposal that the size of the disc produced by this reaction is controlled by conformational changes in apo A-I that results in the all-or-none binding of complete helical (hinged) domains (11). Recently, this hypothesis has been supported by the preliminary studies of apo A-I/POPC micellar subspecies (12).

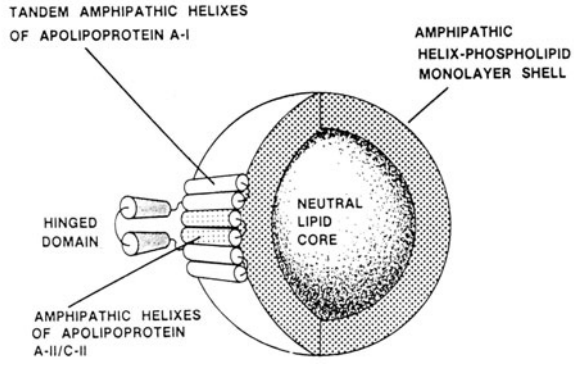


Fig. 10. Model of the "hinged-domain" hypothesis.

The mechanism of subspecies formation in HDL based on the "hinged domain" hypothesis is illustrated in Fig. 10.

Using affinity columns containing anti-apo A-I or anti-apo A-II, nine plasma HDL subpopulations were isolated and characterized using single vertical spin centrifugation (13, 14), electronmicroscopy and nondenaturing gradient gel electrophoresis (8). These were designated as HDL 1, 2, 3, 4, 5, 6, 7a,7b,7c, 8, and 9. The difference in surface area of HDL 4 compared to HDL 3, HDL 5 compared to HDL 4, and HDL 6 compared to HDL 5 averaged 26000 ± 300 A2, the differences in surface area of HDL 7b compared to HDL 7a, and HDL 7c compared to HDL 7b average 3400 ± 100 A^2, while the difference in surface area of HDL 7a compared to HDL 6 was 56000 A^2. This is reminiscent of the results of apo A-I:DMPC recombinants described earlier (11) in which incremental changes in discoidal diameter between several discrete R-2 and R-3 recombinant particles were observed. The quantized changes observed in the HDL population and apo A-I:DMPC discoidal structure could be best explained by the stoichiometric and conformational changes to apo A-I in the hinged-domain.

CONCLUSIONS

1. Class A amphipathic helixes, via the Lys/Arg "snorkel" process, mediate strong lipid affinity without damage or alteration to cell membranes. (Class H and L amphipathic helixes mediate strong lipid affinity but damage or alter cell membranes).

2. Amphipathic helical repeats 2 and 3 represent the major LCAT-activating domain in apo A-I. This domain activates LCAT in a 2-step process:

   Step 1. The amphipathic helix produces a protein-lipid cleft recognized by the active site of LCAT, allowing the enzyme to get at the Sn-2 bond of phosphatidylcholine.

   Step 2. Sequence- and structure-specific glutamyl residues in repeats 2 and 3 create and activated cholesterol intermediate.

3. There are one, or probably two, hinged-domains in apo A-I that have an amphipathic -loop- helical motif. These domains possess two reversible states (lipid-bound and lipid-unbound) that mediate the following:

   a. A low free energy mechanism for reorganization of the amphipathic helical arrays on the edge of discoidal HDL to an array that covers the surface of spheroidal HDL.
   b. Interactions of apo A-I with other apos (E.g., apo A-I).
   c. HDL subspecies (including size quantization).

REFERENCES

1. J. P. Segrest, R. L. Jackson, J. D. Morrisett, and A. M. Gotto, Jr., FEBS Lett., 38:247 (1973).
2. J. P. Segrest, H. De Loof, J. G. Dohlman, C. G. Brouillette, and G. M. Anantharamaiah, Proteins, In press (1990).
3. C -C. Luo, W -H. Li, M. N. Moore, and L. Chan, J. Mol. Biol., 187:325 (1986)
4. G. M. Anantharamaiah, J. L. Jones, C. G. Brouillette, C. F. Schmidt, B. H. Chung, T. A. Hughes, A. S. Bhown, and J.P. Segrest, J.Biol. Chem., 260:10248 (1985).

5. G. M. Anantharamaiah, Synthetic peptide analogs of apolipoproteins, In: Methods of Enzymology, J. P. Segrest and J. J. Albers, eds., Academic Press, New York, 128:627 (1986).
6. R. M. Epand, A. Gawish, M. Iqbal, K. B. Gupta, C. H. Chen, J. P. Segrest, and G. M. Anantharamaiah, J. Biol. Chem., 262:9389 (1987).
7. Y. V. Venkatachalapathi, K. B. Gupta, H. De Loof, J. P. Segrest, and G. M. Anantharamaiah, In: Peptides, ESCOM Press (J. Rivier Ed.) 672 (1990)
8. C. J. Fielding, V. G. Shore, P. E. Fielding, Biophys. Biochem. Res. Commun, 46:1493 (1972).
9. J. J. Albers, J. T. Lin, G. P. Roberts, Artery, 5:61 (1979)
10. G. M. Anantharamaiah, Y. V. Venkatachalapathi, C. G. Brouillette, and J. P. Segrest, Arteriosclerosis, 10:95 (1990).
11. C. G. Brouillette, J. L. Jones, T. C. Ng, H. Kercert, B. H. Chung, and J. P. Segrest, Biochemistry, 23:359 (1984)
12. A. Jonas, K. E. Kezdy, and J. H. Wald, J. Biol. Chem, 264:4818 (1989).
13. M. C. Cheung, J. P. Segrest, J. J. Albers, J. T. Cone, C. G. Brouillette, B. H. Chung, M. Kashyap,, A. Glasscock, and G. M. Anantharamaiah, J. Lipid Res., 28:913 (1987).
14. B. H. Chung, J. P. Segrest M. J. Ray, J. D. Brunzell, J. E. Hokanson, R. M. Krauss, K. Beaudrie, and J. T. Cone, Single vertical spin density gradient ultracentrifugation, In: Methods of Enzymology, J. P. Segrest and J. J. Albers, eds, Academic Press, New York, 128:181 (1986).