Apolipoprotein structural organization in high density lipoproteins: belts, bundles, hinges and hairpins

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Purpose of review

To summarize recent advances towards an understanding of the three-dimensional structures of the apolipoprotein components of HDL with a specific focus on high resolution models of apolipoprotein A-I.

Recent findings

Since the primary sequence was first reported, various models have been advanced for the structure of apolipoprotein A-I, the major protein constituent of HDL, in its lipid-free and lipid-bound forms. Unfortunately, the generation of experimental data capable of distinguishing among the competing models has lagged far behind. However, recent experimental strategies, including X-ray crystallography, applications of resonance energy transfer and mass spectrometry, have combined with sophisticated theoretical approaches to develop three-dimensional structural models of apolipoprotein A-I with previously unavailable resolution.

Summary

The recent synergy of sophisticated computer modeling techniques with hard experimental data has generated new models for apolipoprotein A-I in certain subclasses of HDL produced *in vitro*. The challenge now is to adapt and test these models in the more complex forms of HDL isolated directly from human plasma.

Keywords

apolipoprotein A-I, apolipoproteins, discoidal HDL, HDL, lipid bound, lipid free, spherical HDL, structure, tertiary structure

Curr Opin Lipidol 16:295–300. © 2005 Lippincott Williams & Wilkins.

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Current Opinion in Lipidology 2005, 16:295–300

Abbreviations

apo apolipoprotein
FRET fluorescence resonance energy transfer

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0957-9672

Introduction

It is well established that plasma levels of HDL are inversely proportional to the risk of coronary artery disease [1,2•,3,4]. The most widely recognized HDL function is the movement of cholesterol from the periphery to the liver for catabolism, a process termed reverse cholesterol transport [5,6•]. In addition, there is growing evidence that anti-inflammatory properties of HDL may contribute significant protective effects [4,7••,8••]. Over the past decade, the discoveries of cell surface proteins such as the scavenger receptor BI [9] and the adenosine triphosphate-binding cassette transporter [10•] have helped define the steps of reverse cholesterol transport and fueled a new interest in HDL as a target for coronary artery disease treatment. We are beginning to see the development of drugs designed specifically to raise HDL levels [11••,12].

Although the important players in reverse cholesterol transport are being identified, the molecular events underlying most individual steps are unknown. A major hindrance is a poor understanding of the structure of the main protein constituent of HDL, apolipoprotein (apo) A-I. ApoA-I is a 28 000 M_r, 243 amino acid protein that comprises some 70% of HDL protein mass. It is secreted by the liver and intestine and exists in plasma in three general forms: lipoprotein-unassociated apoA-I, nascent discoidal particles, and mature spherical forms of 70–120 Å in diameter. From an analysis of the amino acid sequence, apoA-I is predicted to contain eight α-helical segments of 22 amino acids and two 11-mer repeats that are frequently separated by proline residues [13]. These amphipathic α-helices contain a hydrophobic face that probably mediates lipid interactions. Unfortunately, the dynamic nature of apoA-I has hampered the study of its tertiary structure. This review covers recent studies of apoA-I organization in the major HDL forms, with special emphasis on the development of high resolution models.

Lipid-free apolipoprotein A-I

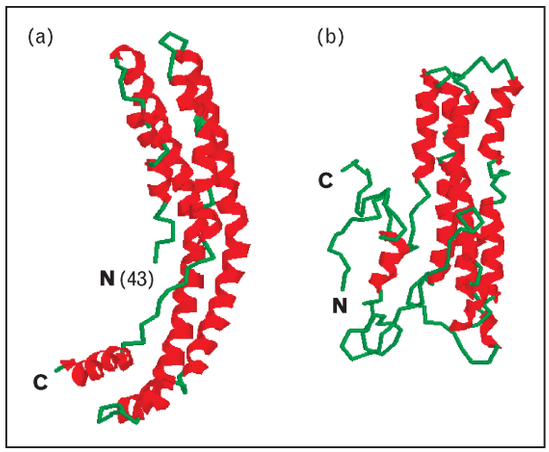
Approximately 5–10% of human plasma apoA-I exists in a lipoprotein-unassociated state. This fraction probably derives from direct secretion by the liver/intestine or dissociation from HDL or triglyceride-rich lipoproteins [14••]. There is currently no strong consensus as to whether the lipoprotein-unassociated apoA-I is completely lipid free or if it contains trace amounts of lipid (lipid poor). Nevertheless, most of the structural studies to date

have focused on apoA-I that has been fully delipidated by organic solvents and studied in aqueous solution. Under these conditions, apoA-I forms a heterogeneous population of oligomers that has frustrated many attempts at deriving a high resolution structure. However, Borhani *et al.* [15] successfully crystallized a fragment of apoA-I that lacked the N-terminal 43 amino acids. The structure at 4 Å resolution showed a ring-shaped assembly of four apoA-I molecules arranged in extended, kinked α-helices. However, because of the missing N-terminus and the tetramerization, the structure appeared to be more applicable to lipid-bound rather than lipid-free apoA-I (see below). Most information on full-length lipid-free apoA-I has thus come from lower resolution approaches such as absorption and fluorescence spectroscopy, analytical ultracentrifugation, limited proteolysis, and monoclonal antibody binding [13,16]. A general consensus from the literature is that lipid-free apoA-I contains at least one domain composed of a bundle of amphipathic α-helices, a motif found in X-ray and NMR structures of other apolipoproteins [17,18]. However, apoA-I is remarkably dynamic. It exhibits characteristics of a 'molten globule' with defined elements of secondary structure, but may contain regions lacking a defined tertiary structure [19,20]. Deletion mutagenesis experiments suggest that apoA-I may contain at least two structural domains. Saito and colleagues [21•,22••] proposed that the N-terminal and central regions are primarily responsible for structural integrity (perhaps comprising a helical bundle), whereas the C-terminus, well known for mediating lipid binding, comprises a separate less organized domain that is poised to interact with lipid. Such a domain structure is reminiscent of apoE, implying that the exchangeable apolipoproteins may share a common organizational theme. However, similar studies of apoA-IV found no evidence of a multidomain organization, and even showed that the lipid binding sequence was not in the C-terminus [23•].

Additional mutation studies have shed light on the tertiary structure of lipid-free apoA-I. Fang *et al.* [24••] suggested that the N and C-termini may interact to stabilize the protein. Furthermore, pH studies indicated that electrostatic interactions, possibly among the helices in a bundle, are also important to the tertiary structure. The same group showed that a peptide mimicking the N-terminal 44 amino acids of apoA-I was unstructured in solution, but became helical in response to lipids [25•].

Although spectroscopic approaches have been useful for understanding the generalities of apoA-I structure and stability, further progress requires a detailed knowledge of which regions physically interact within the molecule. To date, there are two reported atomic-level models of lipid-free apoA-I. The first was built by extracting overlapping regions from the four molecules in the Borhani crystal structure and adding hairpin turns to connect the sequences into a single, contiguous molecule of apoA-I (Fig. 1a) [26]. The result is a bent four helical bundle with a hydrophobic core composed of the non-polar faces of the helices. The model is attractive as it allows for the same interhelical salt bridges postulated in the crystal structure, although they are intramolecular in the monomer. However, the model has some shortcomings when compared with experimental data. For example, it puts the overall helix content at approximately 80%, whereas most experiments estimate the helicity of monomeric apoA-I to be in the range of 45–58%. This discrepancy may result from the non-physiological conditions of the crystallization and the oligomerization [15]. However, the model should be highly useful as a foundation for in-silico molecular dynamics studies designed to simulate more native conditions. A second molecular model was derived from cross-linking experiments combined with sequence threading analysis [27••]. ApoA-I in solution was treated with a lysine-specific cross-linking agent, and joined residues were identified by peptide analysis and mass spectrometry. This resulted in 17 distance constraints for the building of a homology model from template proteins of similar sequence and known three-dimensional structures (Fig. 1b). The model shows a cluster of five helical segments with several intervening regions of random coil. It predicts a reasonable helicity of 56%, and sites of known proteolytic sensitivity correspond closely with predicted breaks in the helical domains. The model also predicts that the N and C-termini are close enough to interact, and provides a basis for the two-domain organization discussed above.

Figure 1. Atomic level models of monomeric lipid-free apolipoprotein A-I



(a) A model created by extracting overlapping regions from the four molecules in the Borhani crystal structure and adding hairpin turns to connect the sequences into a single, contiguous molecule of apolipoprotein (apo) A-I (Brouillette and Harvey, cited in Klon *et al.* [26]). Contains residues 43–243 of apoA-I. (b) A full-length model generated from cross-linking and sequence threading techniques (Silva *et al.* [27••]).

Although both models represent static snapshots of a highly dynamic system, their predictions will be an important context for further experiments.

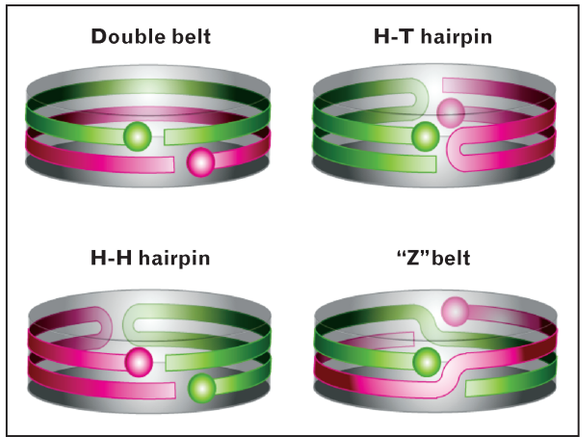
Discoidal HDL

Although of low abundance in plasma, discoidal HDL are critical intermediates between lipid-free/poor apoA-I and mature spherical HDL, which comprise the bulk of circulating particles. Segrest [28] proposed in the late 1970s that the α-helices of apoA-I wrap around the circumference of discoidal HDL with the long axis perpendicular to the acyl chains, i.e. the 'belt' model. Alternatively, it was theorized that the 22 amino acid helical repeats, punctuated by turns, could traverse the bilayer edge parallel to the acyl chains [28]. This 'picket fence' model was favored in the 1980s and early 1990s because of supporting infrared spectroscopy studies [29] and particle geometry arguments [30]. However, the Borhani crystal structure [15] caused a re-evaluation of the model. Although the crystal lacked lipid, the ring shape and absence of hairpin turns implied that apoA-I may prefer a belt-like orientation in lipoproteins. Since then, there have been two studies on true lipid-containing particles that have definitively ruled out the picket fence. The first was methodologically updated infrared experiments by Koppaka *et al.* [31] that contradicted the earlier infrared studies supporting the picket fence. The second was fluorescence depth measurements of probes on each helix that strongly supported a belt model for apoA-I [32] as well as for apoE [33•].

With the question of helical orientation addressed, attention focused on determining the spatial relationships between molecules of apoA-I on a disc. The belt model with the best theoretical support is the 'double belt' model, in which each of two ring-shaped apoA-I molecules wrap around a leaflet in an anti-parallel orientation (Fig. 2) [16,26,34]. Computer analysis predicted a registry between the monomers with similar intermolecular salt bridge connections implied by the crystal structure. An alternative belt model [13] proposed two possible hairpin orientations (Fig. 2) in which each molecule interacts with both leaflets after a turn. This allows similar salt bridge interactions as the double belt, although they are intramolecular in the hairpin. We also proposed a 'Z'-belt orientation that has potential for symmetric, interlocking interactions between the molecules [32].

Distinguishing between the models in Fig. 2 requires methods that provide distance information. Two studies have approached this problem using fluorescence resonance energy transfer (FRET). Li *et al.* [35] demonstrated that fluorescent probes placed in helix 5 of apoA-I could interact in a disc containing two molecules of apoA-I, suggesting a helix 5 to helix 5 contact between the molecules. This argued against the picket fence and

Figure 2. Belt models proposed for apolipoprotein A-I on the edge of discoidal HDL particles containing two molecules of apolipoprotein A-I



Discoidal forms of HDL are postulated to contain a patch of phospholipid/cholesterol bilayer stabilized at its edges by the amphipathic helical domains of apolipoprotein A-I (apoA-I). In each postulated belt model, two protein molecules are shown as belts. The N-terminal 43 residues, which do not fit the typical amphipathic helical pattern of the remaining molecule, are shown as a sphere in each molecule.

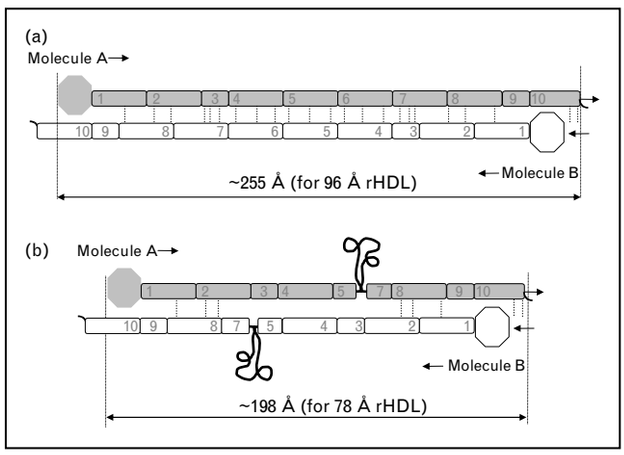
Z-belt orientations, but was consistent with the double belt and hairpin models (assuming the hairpins bend near helix 5). Tricerri *et al.* [36] measured distances at two sites in lipid-bound apoA-I by FRET. They concluded that their data were inconsistent with both the picket fence and double belt models, but could be consistent with a mixture of head-to-head and head-to-tail hairpins. More recently, we applied a cross-linking approach to discoidal HDL particles containing two molecules of apoA-I [37•]. The result was 15 distance constraints that clearly ruled out the picket fence, Z-belt and head-to-tail hairpin models. However, the cross-links were highly consistent with both the double belt and head-to-head hairpin models, providing direct evidence for the salt bridge interactions proposed for both models. The simplest conclusion is that apoA-I can adopt both conformations on otherwise similarly sized particles. With a similar salt bridge potential, there may not be a major thermodynamic advantage for one model over the other. This could explain relatively inefficient energy transfer parameters observed in the studies by Li *et al.* [35] and Tricerri *et al.* [36]. More recent studies within our laboratory have used optimized reconstitution methods to generate a population of apoA-I-containing discs that exclusively exhibit the double belt conformation measured by the cross-linking approach (Silva and Davidson, unpublished data). ApoA-I may thus be able to adopt distinct conformations on different HDL particles.

The hinge domain

Because HDL varies significantly in size, it has been postulated that apoA-I can respond to changes in particle

diameter by folding or unfolding a 'hinge' domain. Various studies have indicated that residues 87–165 [38–41] exhibit conformational flexibility on HDL particles, but the exact mechanism of the transition has been unclear. Two recent studies have taken different approaches to address the hinge domain idea [42••,43•]. Li *et al.* [42••] demonstrated that apoA-I reacts with dimyristoylphosphatidylcholine to generate five distinct discoidal particles with diameters ranging from 98 to 120 Å. Interestingly, the diameters of the two smallest particles were independent of the presence of the N-terminal 43 amino acids of apoA-I, suggesting that a domain further C-terminal could modulate the diameter. However, the N-terminal domain was required to form the three larger particles, implying that short helical domains in the N-terminus could also modify particle diameter. Alternatively, Maiorano *et al.* [43•] measured the exposure of tryptophan residues in each helix of apoA-I to lipid-based quenching agents within discoidal particles. They demonstrated that helices 5, 6 and 7 can swing away from the disc edge when the particle diameter was reduced from 96 to 78 Å. Figure 3 shows one possible model for the response of this domain to particle diameter changes. The clear implication from both studies is that there may be two hinge domains: one in the central region of apoA-I and one in the N-terminus. Perhaps each domain is activated under specific conditions involving particle lipid composition, surface pressure or particle morphology.

Figure 3. A hypothesized 'hinge' domain that may mediate discoidal HDL diameter in the double belt model



Two molecules of apolipoprotein (apo) A-I are represented as either grey or white as if they had been taken off the edge of a discoidal HDL particle and laid flat. (a) The double belt model for a 96 Å reconstituted HDL particle. The registry of the helices is shown in the 5/5 anti-parallel orientation as modeled by Segrest *et al.* [34]. Residues 1–44 are shown as an octagon. Putative salt bridge interactions are represented by the dotted lines. Each amphipathic helical segment is represented as a rectangle and numbered according to the scheme of Roberts *et al.* [44]. (b) A double belt model for the 78 Å particle in which the hinge domain proposed by Maiorano *et al.* [43•] has been removed from the disc edge and represented as a random coil (solid black line).

Spherical HDL

The problem of apoA-I conformation on a spherical particle is more complicated than for a disc. In a sphere, the neutral lipid core allows the surface phospholipid molecules to form a continuous monolayer. The apoA-I helices probably float among the phospholipid molecules with their hydrophobic faces penetrating past the phosphate group to interact with the acyl chains [45]. Without restriction to acyl chains at the disc edge, it is quite possible that the protein could spread out across the particle surface and may not exhibit the same protein–protein contacts found in the discs. There is general agreement that apoA-I in a spherical particle exhibits conformational differences versus discs [40,46–48]. However, it is not clear if these are relatively small adaptations of, say, a modified double belt model, or a completely different spatial arrangement in the spheres. One study has suggested the latter as FRET distances measured in spheres were different to those measured in discs [49]. More experimental distance information is clearly required before distinguishing between these possibilities. The required experiments will be straightforward for reconstituted spheres, but a critical challenge will be to extend studies to the inherently heterogeneous particles obtained from living humans. This will require a significant departure from traditional spectroscopic techniques that depend on a homogeneous population of particles.

Conclusion

Clearly, apoA-I is a dynamic molecule capable of adopting an array of structures along the reverse cholesterol transport pathway. It is the relationship between this structural flexibility and the potential for functional flexibility that makes understanding the apoA-I structure so critical. When one looks at the recent work in this field, it is clear that the major advances were precipitated by the availability of detailed theoretical models providing testable predictions for experimental follow-up. The atomic resolution double belt and picket fence theoretical models prompted focused experiments that have given us a reasonable idea of apoA-I conformation in discoidal particles. Hopefully, we can look forward to similar examinations of the lipid-free apoA-I models in Fig. 1. In addition, continuing efforts need to be made to obtain high resolution crystal or nuclear magnetic resonance structures of HDL apolipoproteins in lipid-free and lipid-bound forms. This may be facilitated by mutagenesis strategies that engineer out the propensity for oligomerization.

Once we have solid information on apoA-I structure in the major HDL subpopulations, we can begin to address other important issues, such as the interaction of apoA-I with other apolipoproteins within HDL and the structural basis of its interactions with cell surface proteins and plasma enzymes. Regarding the latter issue,

an interesting implication of the double belt model is the potential for apoA-I molecules to slide relative to each other [49,50], like thumbwheels on a combination lock. Such a movement could easily change the topology of binding sites for plasma enzymes or cellular binding proteins. One can imagine that some parameter, such as the cholesteryl ester content of an HDL particle, could dictate the conversion of the resident apoA-I molecules between two registries. One registry might be specific for, say, lecithin : cholesterol acyl transferase activation, whereas another might allow interactions with cholesteryl ester transfer protein. This would provide a straightforward way for apoA-I structure to modulate HDL metabolism directly. Although purely hypothetical now, such scenarios provide a fascinating basis for future studies.

Acknowledgements

Cited work performed in the authors' laboratory was supported by grants HL67093 and HL62542 from the National Institutes of Health and a post-doctoral fellowship from the Ohio Valley Affiliate of the American Heart Association. The authors would like to thank Drs Steven Harvey and Christie Brouillette for providing the coordinates for the lipid-free apoA-I model shown in Fig. 1a.

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