CMLS, Cell. Mol. Life Sci. 58 (2001) 885–893
1420-682X/01/070885-09 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Review

Structural studies of discoidal lipoprotein A-I

V. Koppaka

Department of Pharmacology, University of Pennsylvania, 3620 Hamilton Walk, Philadelphia (Pennsylvania 19104-6084, USA), Fax +1 215 573 2236, e-mail: koppaka@pharm.med.upenn.edu

Received 8 September 2000; received after revision 6 December 2000; accepted 4 January 2001

Abstract. Apolipoprotein A-I (apoA-I) is a major exchangeable apolipoprotein of high-density lipoproteins (HDLs), and plays an important role in reverse cholesterol transport. This process involves transport of cholesterol from peripheral tissues to the liver for processing, thereby eliminating excess cholesterol from the body. The function of apoA-I and its interaction with other components of HDL, including lecithin-cholesterol acyltransferase, seems to be closely linked to its structural plasticity. ApoA-I is likely to undergo changes in its structure and orientation between the various HDL subclasses and, therefore, knowledge of the precise structure of apoA-I is essential for understanding its role in the antiatherogenic properties of HDL. This review focuses on the role of apoA-I in reverse cholesterol transport and the work done by various groups to determine the structure of apoA-I in discoidal HDL particles.

Key words. ApoA-I; belt; picket-fence; PATIR-FTIR; orientation.

Introduction

Apolipoprotein A-I(apoA-I) forms the major protein component of high-density lipoproteins (HDLs) comprising about 70% of the total protein. The other main component is apoA-II (20%), with varying amounts of apoC-I, apoC-II, apoC-III, apoD, and apoE comprising the remainder. HDL particles in plasma exist in a heterogeneous population of sizes, shapes, density, and electrophoretic mobility depending upon their lipid and protein composition. Three decades have passed since recognition of the important role that HDLs play in cholesterol efflux from cells. This is the first step in the reverse cholesterol transport pathway, and apoA-I is the principal activator of this process [1, 2]. ApoA-I is therefore closely linked to the antiatherogenic functions of HDLs. It is precisely this feature of apoA-I that has motivated investigations to understand its role in lipid transport and cholesterol homeostasis. However, defining its functional role has been difficult due to the lack of precise knowledge of its structure, especially in its lipid-bound state.

Plasma levels of cholesterol-containing HDL are inversely related to the risk of atherosclerotic coronary vascular disease [3]. Animal studies indicate that plasma HDL or one of its components is pharmacologically active as an antiatherogenic agent [4–6]. Furthermore, interventions aimed at elevating plasma HDL and apoA-I levels in humans and mice appear to protect against atherosclerotic disease [7–10]. ApoA-I has also been shown to be involved in diverse processes outside lipid metabolism and cholesterol homeostasis, displaying antithrombogenic [11–13], antiviral [14, 15], antioxidant [16] and other activities.

ApoA-I and reverse cholesterol transport

Reverse cholesterol transport is a multi-step process that involves the removal of excess cholesterol in peripheral tissues and arterial walls, esterification by means of lecithin-cholesterol acyltransferase (LCAT), and finally transport to the liver for excretion or recycling. ApoA-I

modulates this process by being a preferential acceptor of cellular cholesterol [17], and by increasing the activity of LCAT several-fold [18, 19]. ApoA-I is also involved in transport of LCAT-derived cholesteryl esters to the liver, possibly through receptor-mediated recognition [20].

ApoA-I association with lipids results in three principal forms of HDL particles, the 'lipid-poor' form, the discoidal form, and the spherical. All three forms of apoA-I-associated HDL particles play specific roles in reverse cholesterol transport. Lipid-poor HDL exhibits a slower pre-β electrophoretic mobility and comprises about 2–5% of plasma HDL [21–23]. ApoA-I is often the only protein associated with these particles, and the lipid component consists of roughly equal proportions of lecithin and sphingomyelin [22]. Once bound to small amounts of phospholipid, lipid-poor HDL becomes an acceptor for cholesterol [21, 24]. As this particle becomes rich in its lipid content, it acquires a discoidal shape. This may be accompanied by further changes in both lipid and protein composition. The efflux of cholesterol to the apoA-I-containing HDL particle by diffusion-based and/or receptor-mediated mechanisms depends upon the HDL phospholipid content and composition [21, 22, 25, 26]. Studies have also demonstrated the importance of apolipoprotein composition in the cholesterol-accepting efficiency of HDL, with apoA-II having a negative influence on the efflux [27].

Patients with Tangier disease, an autosomal recessive disorder of lipid metabolism, have a defective ABC1 (ATP-binding cassette transporter 1). In these patients, mature HDL is not formed and lipid-poor apoA-I-containing HDL are therefore rapidly cleared from the plasma [28–30]. This finding suggested that ABC1 mediates or regulates the efflux of cholesterol and phospholipids to HDL and may therefore play an important role in the efflux of cholesterol and phospholipids from cells. ABC1, also called CERP (cholesterol-efflux regulatory protein), is an integral membrane protein whose precise location in the cell has yet to be established. Evidence has also been provided indicating a direct interaction of ABC1 with apoA-I but not HDL, thereby suggesting that lipid-poor apoA-I may be a physiological acceptor for ABC1-mediated lipid efflux [31, 32].

Nascent HDLs are discoidal lipid-apolipoprotein particles less than 10 nm in diameter, usually containing apoA-I, apoA-IV, and apoE. Although the size of these discoidal particles varies depending upon their lipid and protein content and composition, their thickness remains constant and corresponds to a phospholipid bilayer [33]. Discoidal HDL particles can be generated during lipolysis of triglyceride-rich very low density lipoprotein (VLDL) or chylomicrons [34] and are also synthesized by the liver [35, 36]. Interactions with various plasma enzymes and other lipoprotein complexes in the circulation cause these discoidal complexes to become larger and spherical. This remodeling process can be observed in vitro by incubating HDL complexes with LCAT. When discoidal particles with a diameter of 7.9 nm containing phosphatidylcholine, unesterified cholesterol, and two molecules of apoA-I are incubated with LCAT, they increase in size to 8.8 nm and become spherical. The cholesterol on the surface of the discoidal HDL particle after being converted to cholesteryl ester moves to the interior and forms the core of the spherical particle. The bilayer structure for lipids, a characteristic feature of a discoidal particle, is now no longer tenable, and the spherical particle has the lipids resembling more of a micellar structure. Curiously, this transformation seems to be accompanied by an increase in the number of apoA-I molecules per complex from two to three, either by fusion of two discoidal particles or direct incorporation of a lipid-free apolipoprotein [37].

LCAT is an enzyme that catalyzes the esterification of free cholesterol with fatty acid. This reaction takes place on the surface of HDL particles and requires the presence of apoA-I as a cofactor. Although LCAT shows enzymatic activity toward monomeric acyl donors, the physiological substrates are usually the aggregated forms. Discoidal HDL particles containing unesterified or free cholesterol, apoA-I, and phospholipid are considered ideal substrates for LCAT [19, 38]. LCAT is most active at removing an unsaturated acyl chain from the *sn*-2 position of 1,2-acyl phosphatidylcholine and esterifying them to the 3-OH of cholesterol [38]. The lyso-phosphatidyl choline resulting from the action of LCAT diffuses out of the HDL complex and is adsorbed by albumin, while the cholesterol esters formed migrate to the interior of the discoidal particle. This causes a change in shape from a discoidal to a spherical particle. In the absence of cholesterol, LCAT has phospholipase A_2 activity. Both these reactions of LCAT on lipoprotein particles and on lipid vesicles are known to be dramatically stimulated by apoA-I [39]. The enhancement of the catalytic rate has been proposed to be due to a conformational change that facilitates access of the active site in LCAT to the substrate [38], probably through a direct interaction between the enzyme and the cofactor [40–42]. ApoA-I is therefore expected to accommodate or adjust to changes in composition of both lipid and protein components and changes in shape of the HDL particle from a discoidal to a spherical particle. These changes may require a conformational flexibility in the overall structure of apoA-I.

Cholesterol esters in the spherical HDL complexes have two principal fates [36]. They may be extracted by scavenger-receptor B1 (SR-B1) in the liver and various steroidogenic tissues [43, 44]. Alternatively, they may be exchanged for triglycerides in circulating VLDLs by means of the cholesterol ester transfer protein (CETP). As VLDLs accumulate cholesterol, they become low-density lipoproteins (LDLs) and bind to LDL receptors for re-uptake by the liver or other tissues.

Structural characterization of ApoA-I

Human apoA-I originates in both the liver and small intestine, initially as pre-pro-apoA-I. This undergoes proteolytic processing to form the 243-amino-acid apoA-I [45]. One of the main structural hallmarks of this polypeptide is the presence of a series of 11- and 22-residue amphipathic α helices as derived initially from the analysis of the primary sequence [46]. α helices are the main conformational motif for HDL apolipoproteins, and the helical content increases substantially when apolipoproteins bind to phospholipids [47, 48].

A variety of techniques and strategies have been employed to study apoA-I structure on HDL particles. However, a detailed structural analysis with the traditional methods of structural biology has been difficult because of the heterogeneity in the HDL population and the structural plasticity of apoA-I. Biophysical studies and sequence analyses initially suggested that apoA-I may form a series of short amphiphilic helices [49–56]. Studies of HDL with neutron scattering, X-ray scattering, cryo-electron microscopy, and atomic force microscopy have pointed to the location of apoA-I in complexes at the disc edge and have defined the size and shape of discoidal complexes as being consistent with a bilamellar organization of lipid [57–61]. A number of expression systems have been used to express recombinant protein with specific deletions or mutations of specific amino acid residues [62–66]. These studies have been useful in determining the structure-function relationship of apo A-I. None of these techniques, however, could verify the conformation or orientation of apoA-I in lipoprotein complexes under native conditions.

Most of the structural studies have been done using discoidal HDL particles that are reconstituted by either a spontaneous reaction of apoA-I with phospholipid vesicles or by detergent dialysis after all components have been solubilized with a suitable detergent and the particles finally isolated by gel filtration or ultra-centrifugation techniques. Jonas [67] has reviewed in detail the methods for reconstituting HDL complexes. These methods have been used to yield a homogeneously sized population of reconstituted discoidal HDL species, which depend not only upon the initial molar ratio of protein and lipid, but also on the phospholipid composition and physical state of the lipid [35, 67–70].

Orientation of apoA-I on discoidal particles

Belt versus picket-fence model

A model for the amphiphilic helical structure of lipid-binding proteins was first suggested by Segrest and coworkers in 1974 [71]. According to this model, the apoA-I-containing lipid particle has a flattened disc-shaped structure comprised of a single phospholipid bilayer. The helical region of the protein has a polar face that is exposed to the polar ends of the phospholipid or the aqueous phase, and a non-polar face that is buried in the non-polar region of the lipid milieu surrounding the lipid core. This was called the 'bicycle tire' model based on the assumption that the helices would orient themselves tangential to the disc edge in the plane of the disc, thereby forming a protective strip around the disc [59, 72]. Since then, two models have been proposed for the orientation of apoA-I on discoidal HDL particles, the belt model, in which the helices are oriented perpendicular to the lipid acyl chains [72], and the 'picket-fence' model, in which the helical segments are oriented parallel to the lipid acyl chains [73], (fig. 1). In both models however, the protein forms the outer rim of the phospholipid disk.

Using HDL recombinants, Tall and coworkers [73] suggested that the helices of the apoprotein are located at the disc edge, thereby forming an annulus around the perimeter of the disc. They proposed a picket-fence-like orientation of the helices in their model. This model gained support based on the dimensions of reconstituted discoidal HDL and changes in helical content of lipid-bound apoA-I (obtained from circular dichroism measurements) as a function of lipid/protein ratio, and the assumption that major HDL particle classes changed by increments of one apoA-I and not two apoA-I, in which case explaining the belt or bicycle tire model for orientation of the helices would be difficult [67, 69]. Furthermore, based on the observation that apoA-I is punctuated by prolines at regular intervals of 22-amino-acid residues, and the assumption that these prolines mark the beginnings of α helices, the helices of apoA-I in a discoidal HDL particle, were suggested as being oriented parallel to the phospholipid acyl chains in the bilayer [69, 74].

The earliest experimental evidence for the orientation of apoA-I on discoidal HDL complexes came from polarized attenuated total internal reflection-Fourier-transform infrared (PATIR-FTIR) spectroscopy [75, 76]. These experiments were performed by drying lipoprotein complexes onto the crystal surface and partially rehydrating by exposure to water vapor. The results were interpreted to mean that the lipid in nascent HDL complexes formed a bilamellar disc, and that the α helices of apoA-

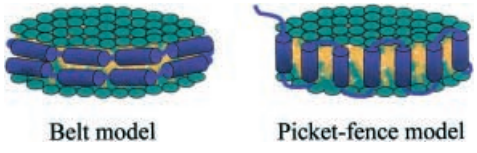

Figure 1. Representation of the two models that have been proposed for the orientation of the amphipathic α helices of apoA-I on discoidal complexes. The blue cylinders represent the 22- and 11-residue repeating amphipathic α-helical units of apoA-I oriented parallel (belt) and perpendicular (picket fence) to the phospholipid bilayer surface.

I aligned themselves parallel to the lipid acyl chains (fig. 1). This 'picket-fence' model became widely accepted, and detailed theoretical studies of this model have been published [77, 78].

Nuclear magnetic resonance studies were carried out for a short segment of apoA-I comprising residues 166–185 corresponding to α-helical repeats 6 and 7, in the presence of detergents or phospholipid micelles by Wang and coworkers [56]. These studies showed that in the presence of micelles, both repeats are α-helical, with a hinge at Pro165 between the two helices. The peptide thus seems to adopt a helix-hinge-helix motif that straddles the micelle. Although these results are frequently cited in support of the picket-fence model, no interhelical interactions were observed between the two helices, as would be expected for a picket-fence orientation. It is important to note that these studies were carried out on micellar structures and not discoidal particles containing physiologically relevant phospholipids. The orientation of apoA-I may change on a spherical HDL particle compared to the discoidal particle.

Experimental evidence suggesting that apoA-I helices may instead be oriented parallel to the membrane surface came from crystallographic studies of a lipid-free amino-terminal truncation mutant of human apoA-I [79], in which residues 1–43 were replaced by an N-terminal Met residue, apoΔ(1–43)A-I. In this structure, four molecules were arranged as pairs of antiparallel dimers adopting an elliptical and curved shape that places the N and C termini of apoΔ(1–43)A-I only 23 Å apart. Rather than a series of short helical segments, apoΔ(1–43)A-I formed a near-continuous helical ring reminiscent of the 'bicycle tire' or 'belt' model [59, 72]. This crystal structure might have been dismissed as an artifact due to the truncation, or to the absence of lipid. However, the discoidal lipoprotein complexes formed with apoΔ(1–43)A-I exhibited physical properties that were in many ways identical to those exhibited by lipoprotein complexes made from full-length apoA-I [64]. Subsequent sedimentation velocity studies indicated that both apoA-I and apoΔ(1–43)A-I formed long extended helices rather than short helical segments in the absence of lipid, which was interpreted as more consistent with a 'belt model' (fig. 1) for the structure of lipoprotein A-I [80, 81].

To examine if this model holds for the orientation of apoA-I and apoΔ(1–43)A-I in the presence of a lipid, an advanced form of PATIR-FTIR spectroscopy (fig. 2) was employed [82]. This technique had features that were not available during the measurements that were done earlier on full-length LpA-I complexes [83–85], both in terms of instrumentation and data analysis [84, 86, 87]. The most significant advance was that the complexes could be studied in their native condition in an aqueous medium, without denaturing the protein or disrupting the complexes. In the earlier studies, the complexes had to be dried onto the crystal surface as a multi-bilayer film and partially rehydrated by exposure to water vapor [75, 76], thereby losing control over ionic strength and pH. More importantly, however, the stability of folded proteins and lipoprotein complexes depends to a large extent on the hydrophobic effect, and there can be no hydrophobic effect in the absence of water. Therefore, drying the complexes – even for a short interval – risks denaturing the proteins and/or disrupting the complexes. Indeed, dried preparations studied by PATIR-FTIR spectroscopy yield results that differ from fully hydrated lipid membranes in ways that suggest denaturation and/or aggregation [88].

The design of this advanced PATIR-FTIR technique permits the acquisition of infrared spectra from only material that is adsorbed to the phospholipid monolayer and not from unadsorbed material floating in the aqueous buffer subphase. A variety of information about lipid-protein interaction can be obtained from the resulting infrared spectra. The shape and position of the peptide/protein amide band yields information about the conformation of the peptide, and the intensity/integrated area of a band can be used to determine the concentration of the material bound to the membrane surface. Polarization spectra, collected by orienting the polarizer parallel or perpendicular to the incident beam of light, can be used to determine the order parameters. These order parameter values in turn yield information about the orientation of lipids comprising the monolayer and proteins/peptides that adsorb and/or insert into the membrane monolayer.

PATIR-FTIR spectroscopy thus allowed the reconstituted apoA-I complexes to be oriented on a monolayer in a continuously stirred aqueous environment, in their native state. Under these conditions, the lipid in discoidal LpA-I and LpΔ(1–43)A-I complexes is clearly oriented as in a bilayer membrane with its surface parallel to the monolayer support. The protein has an α-helical conformation, and protein helices are oriented perpendicular to the lipid acyl chains and parallel to the surface of the lipid disc [82]. These are all of the essential features of the belt model (fig. 1). ApoA-I and apoΔ(1–43)A-I were further demonstrated to exhibit no significant difference in conformation or orientation in their respective complexes, which is consistent with earlier biophysical studies [70]. These studies were done on apoA-I complexes in their native conditions and, thus, unambiguously supported the belt model for the orientation of the apoA-I helices on the bilayer disc.

Further evidence for the belt model

Since the publication of results from the advanced PATIR-FTIR technique, supporting evidence for the belt model has come from three other laboratories. Using computer modeling techniques, Segrest and coworkers [89] have developed a detailed model for a discoidal HDL

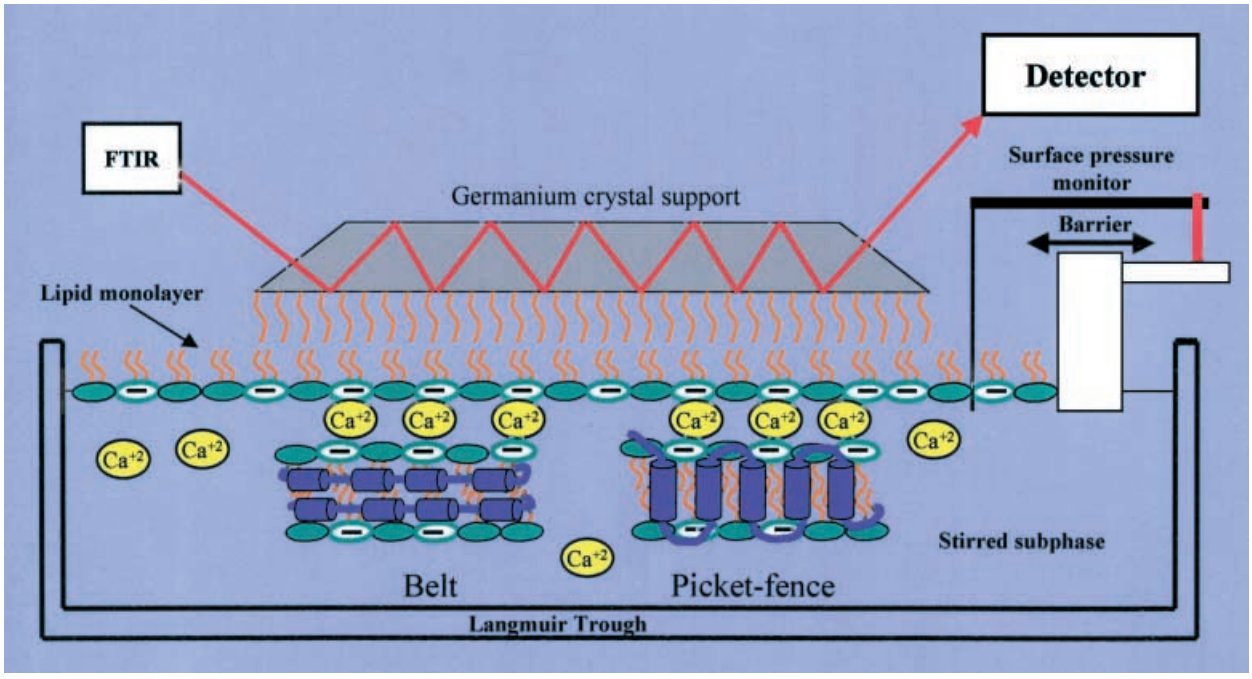

Figure 2. Scheme of the PATIR-FTIR experiment (not to scale) with a representation of the reconstituted lipoprotein complexes. A phospholipid monolayer composed of 80 mole% dimyristoylphosphatidycholine and 20 mole% dimyristoylphosphatidylserine was applied to a germanium crystal treated with octadecyltrichlorosilane. The monolayer formed at the air-water interface on a Langmuir trough was compressed to a surface pressure of 38 dyn/cm by movement of the barrier, before applying to the crystal surface. Infrared light from a commercial FTIR spectrometer was polarized before directing into the crystal, where it makes several internal reflections and is directed on to an external detector. At the site of each internal reflection, only material on the crystal surface absorbs light from the infrared beam producing an absorption spectrum. Material more than a few micrometers away does not contribute to a spectrum. The complexes exhibited a spectroscopic signal only when calcium is present in the subphase buffer, suggesting that calcium forms a bridge between the negatively charged seryl headgroups in the complexes and the monolayer. The lack of signal in the absence of calcium also suggested that there was no free protein in the discoidal complexes. The lipoprotein complexes remain in the continuously stirred buffer subphase throughout an experiment. The relative orientation of the components in the complexes on the crystal surface was determined from the relative absorption intensities of the light polarized parallel and perpendicular to the crystal surface [86, 87]. The blue cylinders represent the 22- and 11-residue repeating amphipathic α-helical units of apoA-I oriented parallel (belt) and perpendicular (picket fence) to the phospholipid bilayer surface.

particle consisting of two apoA-I molecules wrapped 'beltwise' around a patch of 161 lipid molecules arranged in the form of a bilayer. This simulation produced a planar, continuous, amphipathic α-helical torus around the lipid; the C-terminal domain of each apoA-I molecule formed a curved, amphipathic α-helical ring with the concave inner face being completely hydrophobic and surrounding the 80 to 85-Å-diameter phospholipid patch. The outer diameter of the discoid particle was 100–105 Å. A remarkable feature of this model that lends strongest support to the belt model is that there is a perfect alignment of the 20 interhelix salt-bridge pairs. The model, therefore, preserves the nearly perfect correspondence of acidic and basic residues seen in the apoΔ(1–43)A-I crystal structure, so that salt-bridges can form between a pair of apoA-I molecules oriented antiparallel to each other. Other noteworthy points of this model are that the point mutations reported for apoA-I are asymmetrically distributed in a manner consistent with the vulnerabilities expected of this model and, finally, the two cysteine-containing human apoA-I mutants, apoA-I(R173C)$_{Milano}$ and apoA-I(R151C)$_{Paris}$, have their disulfide links located at a position which is most favorable for a belt conformation. This would seem unlikely in a picket-fence model. It is also difficult to imagine the disulfide-linked apoA-I dimer having the picket-fence conformation keeping in mind the energy constrains due to the exposure of hydrophobic domains of the protein in a picket-fence model [90]. Klon and coworkers [91] have now proposed models for the binding of these two naturally occurring mutants of human apoA-I to lipid in discoidal HDL particles. These models are consistent with experimental observations that reconstituted HDL particles containing apoA-I$_{Milano}$ and apoA-I$_{Paris}$ are very similar in size and shape to reconstituted HDL particles containing wild-type apoA-I [92, 93]. The two monomers of the mutant bind the lipid in an antiparallel fashion, with the long axis of their helical repeats oriented parallel to the bilayer surface (belt conformation). This model proposed for mutant apoA-I differs from that proposed for a wild-type apoA-I in the helix-helix registration [89]. This is a result of rotation of the two monomers to bring the two cysteine

residues to a position favorable for forming a covalent bond.

Evidence from two other laboratories in support of the orientation of the α-helical repeats in apoA-I parallel to the membrane bilayer comes from employing fluorescence spectroscopy techniques. Maiorano and Davidson [94] used fluorescence quenching, and Li and coworkers [95] used fluorescence resonance energy transfer techniques.

Maiorano and Davidson [94] have measured the position of tryptophan residues in helix 4 of single-tryptophan mutants of human apoA-I, with respect to a phospholipid bilayer, in reconstituted HDL particles. Using nitroxide spin labels at different depths in the membrane as quenchers of tryptophan fluorescence, they determined by parallax analysis that Trp108 and Trp115 on helix 4 of apoA-I were located at similar depths of the bilayer. This is consistent with the belt model, where the helix is oriented perpendicular to the lipid acyl chain. The same observation was made for reconstituted HDL containing two, three and greater than four molecules of apoA-I per particle. One would expect the tryptophan residues to be located at different depths if there were a picket-fence orientation. Although this work pertains to only helix 4 of the apoA-I molecule, it strongly suggests that apoA-I helices may be oriented parallel to the disc surface.

Li and coworkers [95] fluorescently labeled apoA-I mutants in which glutamine-132 had been mutated to cysteine. The glutamine-132 residue is located in repeat 5 of the amphipathic helix, and according to the belt model, and as predicted by the crystal structure studies [80], this residue in an apoA-I monomer should lie within 16 Å from the glutamine-132 residue of the other monomer. According to the picket-fence model, these residues should be around 104 Å apart. To distinguish between these models, the cysteine in Q132C apoA-I was labeled either with fluorescein (donor) or tetramethyl rhodamine (acceptor). Fluorescence energy transfer between the probes in the two apoA-I monomers on reconstituted HDL particles suggested that the acceptor and donor probes in the monomers were about 30–35 Å apart from each other. These studies also provided additional evidence for belt conformation by demonstrating that the rhodamine dimers in the two apoA-I monomers are approximately 6 Å apart from each other. Although these experiments unequivocally support the belt model, to distinguish between a conformation where an apoA-I monomer can fold back on itself, in the middle of helix 5 to adopt a hairpin-like structure, and an extended belt conformation is difficult from these studies.

Unanswered questions

Several structural questions have yet to be answered unequivocally. For example, how does the structure of apoA-I change when discoidal HDL particles change to mature spherical HDL particles? In addition, what is the structure of a discoidal complex containing three apoA-I molecules? One mechanism proposed for disc formation suggests that apoA-I can adopt a hairpin structure, in which a monomer can fold back on itself in the middle of helix 5, with interhelical interactions still analogous to those present in the crystal structure. This arrangement could wrap around the disc edge and accommodate any of the stoichiometries observed [80]. Although this is quite plausible, experimental evidence for this structure is still lacking.

Determining the structural rearrangements by which apoA-I adjusts to profound changes in HDL particle size and shape is critical. This will help toward a better understanding of the mechanism of reverse cholesterol transport and LCAT activation and, therefore, cholesterol homeostasis.

1 Glomset J. A. (1968) The plasma lecithins:cholesterol acyltransferase reaction. J. Lipid Res. **9:** 155–167
2 Scanu A. M. and Wisdom C. (1972) Serum lipoproteins structure and function. Ann. Rev. Biochem. **41:** 703–730
3 Genest J. J., Marcil M., Denis M. and Yu L. (1999) High density lipoproteins in health and in disease. J. Inves. Med. **47:** 31–42
4 Badimon J. J., Badimon L. and Fuster V. (1990) Regression of atherosclerotic lesions by high density lipoprotein plasma fraction in the cholesterol-fed rabbit. J. Clin. Invest. **85:** 1234–1241
5 Schultz J. R. and Rubin E. M. (1994) The properties of HDL in genetically engineered mice. Curr. Opin. Lipidol. **5:** 126–137
6 Miyazaki A., Sakuma S., Morikawa W., Takiue T., Miake F., Terano T. et al. (1995) Intravenous injection of rabbit apolipoprotein A-I inhibits the progression of atherosclerosis in cholesterol-fed rabbits. Arterioscle. Thromb. Vasc. Biol. **15:** 1882–1888
7 Canner P. L., Berge K. G., Wenger N. K., Stamler J., Friedman L., Prineas R. J. et al. (1986) Fifteen year mortality in Coronary Drug Project patients: long-term benefit with niacin. J. Am. Coll. Cardiol. **8:** 1245–1255
8 Frick M. H., Elo O., Haapa K., Heinonen O. P., Heinsalmi P., Helo P. et al. (1987) Helsinki Heart Study: primary-prevention trial with gemfibrozil in middle-aged men with dyslipidemia. Safety of treatment, changes in risk factors, and incidence of coronary heart disease. N. Engl. J. Med. **317:** 1237–1245
9 Rubin E. M., Krauss R. M., Spangler E. A., Verstuyft J. G. and Clift S. M. (1991) Inhibition of early atherogenesis in transgenic mice by human apolipoprotein AI. Nature, **353:** 265–267
10 Duverger N., Kruth H., Emmanuel F., Caillau, J. M., Viglietta Castro G. et al. (1996) Inhibition of atherosclerosis development in cholesterol-fed human apolipoprotein A-I-transgenic rabbits. Circulation **94:** 713–717
11 Saku K., Ahmad M., Glas-Greenwalt P. and Kashyap M. L. (1985) Activation of fibrinolysis by apolipoproteins of high density lipoproteins in man. Thromb. Res. **39:** 1–8
12 Epand R. M., Stafford A., Leon B., Lock P. E., Tytler E. M., Segrest J. P. et al. (1994) HDL and apolipoprotein A-I protect erythrocytes against the generation of procoagulant activity. Arterioscler. Thromb. **14:** 1775–1783
13 Naqvi T. Z., Shah P. K., Ivey P. A., Molloy M. D., Thomas A. M., Panicker S. et al. (1999) Evidence that high-density lipoprotein cholesterol is an independent predictor of acute

platelet-dependent thrombus formation. Am. J. Cardiol. **84:** 1011–1017
14 Srinivas R. V., Birkedal B., Owens R. J., Anantharamaiah G. M., Segrest J. P. and Compans R. W. (1990) Antiviral effects of apolipoprotein A-I and its synthetic amphipathic peptide analogs. Virology **176:** 48–57
15 Owens R. J., Anantharamaiah G. M., Kahlon J. B., Srinivas R. V., Compans R. W. and Segrest J. P. (1990) Apolipoprotein A-I and its amphipathic helix peptide analogs inhibit human immunodeficiency virus-induced syncytium formation. J. Clin. Invest. **86:** 1142–1150
16 Kunitake S. T., Jarvis M. R., Hamilton R. L. and Kane J. P. (1992) Binding of transition metals by apolipoprotein A-I-containing plasma lipoproteins: inhibition of oxidation of low density lipoproteins. Proc. Nat. Acad. Sci. USA **89:** 6993–6997
17 Rothblat G. H., Mahlberg F. H., Johnson W. J. and Phillips M. C. (1992) Apolipoproteins, membrane cholesterol domains, and the regulation of cholesterol efflux. J. Lipid Res. **33:** 1091–1097
18 Mahley R. W., Innerarity T. L., Rall S. C. J. and Weisgraber K. H. (1984) Plasma lipoproteins: apolipoprotein structure and function. J. Lipid Res. **25:** 1277–1294
19 Jonas A. (1991) Lecithin-cholesterol acyltransferase in the metabolism of high-density lipoproteins. Biochim. Biophys. Acta, 1084, 205–220
20 Morrison J. R., McPherson G. A. and Fidge N. H. (1992) Evidence for 2 sites on rat-liver plasma-membranes which interact with high-density lipoprotein(3). J. Biol. Chem. **267:** 13205–13209
21 Rothblat G. H., Llera-Moya M. de la, Atger V., Kellner W., Williams D. L. and Phillips M. C. (1999) Cell cholesterol efflux: integration of old and new observations provides new insights. J. Lipid Res. **40:** 781–796
22 Fielding C. J. and Fielding P. E. (1995) Molecular physiology of reverse cholesterol transport. J. Lipid Res. **36:** 211–228
23 Kunitake S. T., Mendel C. M. and Hennessy L. K. (1992) Interconversion between apolipoprotein A-I-containing lipoproteins of pre-beta and alpha electrophoretic mobilities. J. Lipid Res. **33:** 1807–1816
24 Castro G. R. and Fielding C. J. (1988) Early incorporation of cell-derived cholesterol into pre-beta-migrating high-density lipoprotein. Biochemistry **27:** 25–29
25 Fournier N., Paul J. L., Atger V., Cogny A., Soni T., Llera-Moya M. de la, et al. (1997) HDL phospholipid content and composition as a major factor determining cholesterol efflux capacity from Fu5AH cells to human serum. Arterioscler. Thromb. Vasc. Biol. **17:** 2685–2691
26 Jian B., Llera-Moya M. de la, Royer L., Rothblat G., Francone O. and Swaney J. B. (1997) Modification of the cholesterol efflux properties of human serum by enrichment with phospholipid. J. Lipid Res. **38:** 734–744
27 Chiesa G., Parolini C., Canavesi M., Colombo N., Sirtori C. R., Fumagalli R. et al. (1998) Human apolipoproteins A-I and A-II in cell cholesterol efflux: studies with transgenic mice. Arterioscler. Thromb. Vasc. Biol. **18:** 1417–1423
28 Brooks-Wilson A., Marcil M., Clee S. M., Zhang L. H., Roomp K., Dam M. van et al. (1999) Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. Nat Genet. **22:** 336–345
29 Bodzioch M., Orso E., Klucken J., Langmann T., Bottcher A., Diederich W. et al. (1999) The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease [see comments]. Nat. Genet. **22:** 347–351
30 Rust S., Rosier M., Funke H., Real J., Amoura Z., Piette J. C. et al. (1999) Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. Nat. Genet. **22:** 352–355
31 Lawn R. M., Wade D. P., Garvin M. R., Wang X., Schwartz K., Porter J. G. et al. (1999) The Tangier disease gene product ABC1 controls the cellular apolipoprotein-mediated lipid removal pathway. J. Clin. Invest. **104:** R25–R31
32 Wang N, Silver D. L., Costet P. and Tall A. R. (2000) Specific binding of ApoA-I, enhanced cholesterol efflux, and altered plasma membrane morphology in cells expressing ABC1. J. Biol. Chem. **274:** 33053–33058 (GENERIC)
33 Hamilton R. L., Williams M. C., Fielding C. J. and Havel R. J. (1976) Discoidal bilayer structure of nascent high density lipoproteins from perfused rat liver. J. Clin. Invest. **58:** 667–680
34 Eisenberg S. (1984) High density lipoprotein metabolism. J. Lipid Res. **25:** 1017–1058
35 Jonas A. (1984) A review of plasma apolipoprotein A-I interactions with phosphatidylcholines. Exp. Lung Res. **6:** 255–270
36 Tall A. R. (1998) An overview of reverse cholesterol transport. Eur. Heart J. **19: (suppl A):** A31–A35
37 Liang H. Q., Rye K. A. and Barter P. J. (1996) Remodelling of reconstituted high density lipoproteins by lecithin: cholesterol acyltransferase. J. Lipid Res. **37:** 1962–1970
38 Jonas A. (1998) Regulation of lecithin cholesterol acyltransferase activity. Prog. Lipid Res. **37:** 209–234
39 Fielding C. J., Shore V. G. and Fielding P. E. (1972) A protein cofactor of lecithin: cholesterol acyltransferase. Biochem. Biophys. Res. Commun. **46:** 1493–1498
40 Sparks D. L., Frank P. G., Braschi S., Neville T. A. and Marcel Y. L. (1999) Effect of apolipoprotein A-I lipidation on the formation and function of pre-beta and alpha-migrating LpA-I particles. Biochemistry **38:** 1727–1735
41 Jin L., Shieh J. J., Grabbe E., Adimoolam S., Durbin D. and Jonas A. (1999) Surface plasmon resonance biosensor studies of human wild-type and mutant lecithin cholesterol acyltransferase interactions with lipoproteins. Biochemistry **38:** 15659–15665
42 Sviridov D., Hoang A., Sawyer W. H. and Fidge N. H. (2000) Identification of a sequence of apolipoprotein A-I associated with the activation of lecithin : cholesterol acyltransferase. J. Biol. Chem. **275:** 19707–19712
43 Acton S., Rigotti A., Landschulz K. T., Xu S., Hobbs H. H. and Krieger M. (1996) Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. Science **271:** 518–520
44 Trigatti B., Rayburn H., Vinals M., Braun A., Miettinen H., Penman M. (1999) Influence of the high density lipoprotein receptor SR-BI on reproductive and cardiovascular pathophysiology. Proc. Nat. Acad. Sci. USA **96:** 9322–9327
45 Brewer H. B. J., Fairwell T., LaRue A., Ronan R., Houser A. and Bronzert T. J. (1978) The amino acid sequence of human APOA-I, an apolipoprotein isolated from high density lipoproteins. Biochem. Biophys. Res. Commun. **80:** 623–630
46 McLachlan A. D. (1977) Repeated helical pattern in apolipoprotein-A-I. Nature **267:** 465–466
47 Lux S. E., Hirz R., Shrager R. I. and Gotto A. M. (1972) The influence of lipid on the conformation of human plasma high density apolipoproteins. J. Biol. Chem. **247:** 2598–2606
48 Sparrow J. T., Gotto A. M. J. and Morrisett J. D. (1973) Chemical synthesis and biochemical properties of peptide fragments of apolipoprotein-alanine. Proc. Nat. Acad. Sci. USA **70:** 2124–2128
49 Brouillette C. G., Jones J. L., Ng T. C., Kercret H., Chung B. H. and Segrest J. P. (1984) Structural studies of apolipoprotein A-I/phosphatidylcholine recombinants by high-field proton NMR, nondenaturing gradient gel electrophoresis, and electron microscopy. Biochemistry **23:** 359–367
50 Boguski M. S., Elshourbagy N., Taylor J. M. and Gordon J. I. (1985) Comparative analysis of repeated sequences in rat apolipoproteins A-I, A-IV, and E. Proc. Nat. Acad. Sci. USA **82:** 992–996
51 Luo C. C., Li W. H., Moore M. N. and Chan L. (1986) Structure and evolution of the apolipoprotein multigene family. J. Mol. Biol. **187:** 325–340

52 De Loof H., Rosseneu M., Yang C. Y., Li W. H., Gotto A. M. Jr. and Chan L. (1987) Human apolipoprotein B: analysis of internal repeats and homology with other apolipoproteins. J. Lipid Res. **28:** 1455–1465
53 Wald J. H., Krul E. S. and Jonas A. (1990) Structure of apolipoprotein A-I in three homogeneous, reconstituted high density lipoprotein particles. J. Biol. Chem. **265:** 20037–20043
54 Nolte R. T. and Atkinson D. (1992) Conformational analysis of apolipoprotein A-1 and E-3 based on primary sequence and circular dichroism. Biophys. J. **63:** 1221–1239
55 Segrest J. P., Jones M. K., De Loof H., Brouillette C. G., Venkatachalapathi Y. V. and Anantharamaiah G. M. (1992) The amphipathic helix in the exchangeable apolipoproteins: a review of secondary structure and function. J. Lipid Res. **33:** 141–166
56 Wang G., Sparrow J. T. and Cushley R. J. (1997) The helix-hinge-helix structural motif in human apolipoprotein A-I determined by NMR spectroscopy. Biochemistry **36:** 13657–13666
57 Forte T. M., Nichols A. V., Gong E. L., Levy R. I. and Lux S. (1971) Electron microscopic study on reassembly of plasma high density apoprotein with various lipids. Biochim. Biophys. Acta **248:** 381–386
58 Andrews A. L., Atkinson D., Barratt M. D., Finer E. G., Hauser H., Henry R. et al. (1976) Interaction of apoprotein from porcine high-density lipoprotein with dimyristoly lecithin. 2. Nature of lipid-protein interaction. Eur. J. Biochem. **64:** 549–563
59 Wlodawer A., Segrest J. P., Chung B. H., Chiovetti R. J. and Weinstein J. N. (1979) High-density lipoprotein recombinants: evidence for a bicycle tire micelle structure obtained by neutron scattering and electron microscopy. FEBS Lett. **104:** 231–235
60 Carlson J. W., Jonas A. and Sligar S. G. (1997) Imaging and manipulation of high-density lipoproteins. Biophys. J. **73:** 1184–1189
61 Antwerpen R. van, Chen G. C., Pullinger C. R., Kane J. P., LaBelle M., Krauss R. M. et al. (1997) Cryo-electron microscopy of low density lipoprotein and reconstituted discoidal high density lipoprotein: imaging of the apolipoprotein moiety. J. Lipid Res. **38:** 659–669
62 Bergeron J., Frank P. G., Emmanuel F., Latta M., Zhao Y., Sparks D. L. et al. (1997) Characterization of human apolipoprotein A-I expressed in *Escherichia coli.* Biochim. Biophys. Acta **1344:** 139–152
63 Frank P. G., Bergeron J., Emmanuel F., Lavigne J. P., Sparks D. L., Denefle P. et al. (1997) Deletion of central alpha-helices in human apolipoprotein A-I: effect on phospholipid association. Biochemistry **36:** 1798–1806
64 Rogers D. P., Brouillette C. G., Engler J. A., Tendian S. W., Roberts L., Mishra V. K. et al. (1997) Truncation of the amino terminus of human apolipoprotein A-I substantially alters only the lipid-free conformation. Biochemistry **36:** 288–300
65 Davidson W. S., Hazlett T., Mantulin W. W. and Jonas A. (1996) The role of apolipoprotein AI domains in lipid binding. Proc. Nat. Acad. Sci. USA **93:** 13605–13610
66 Sorci-Thomas M., Curtiss L., Parks J., Thomas M., Kearns M. and Landrum M. (1998) The hydrophobic face orientation of apolipoprotein A-I amphipathic helix domain 143–164 regulates lecithin:cholesterol acyltransferase activation. J. Biol. Chem. **273:** 11776–11782
67 Jonas A. (1986) Reconstitution of high-density lipoproteins. Methods in Enzymol. **128:** 553–582
68 Matz, C. E. and Jonas, A. (1982) Micellar complexes of human apolipoprotein A-I with phosphatidylcholines and cholesterol prepared from cholate-lipid dispersions. J. Biol. Chem. **257:** 4535–4540
69 Jonas A., Kezdy K. E. and Wald J. H. (1989) Defined apolipoprotein A-I conformations in reconstituted high density lipoprotein discs. J. Biol. Chem. **264:** 4818–4824
70 Rogers D. P., Brouillette C. G., Engler J. A., Tendian S. W., Roberts L., Mishra V. K. et al. (1997) Truncation of the amino terminus of human apolipoprotein A-I substantially alters only the lipid-free conformation. Biochemistry **36:** 288–300
71 Segrest J. P., Jackson R. L., Morrisett J. D. and Gotto A. M. Jr (1974) A molecular theory of lipid-protein interactions in the plasma lipoproteins. FEBS Lett. **38:** 247–258
72 Segrest J. P. (1977) Amphipathic helixes and plasma lipoproteins: thermodynamic and geometric considerations. Chem Phys. Lipids **18:** 7–22
73 Tall A. R., Small D. M., Deckelbaum R. J. and Shipley G. G. (1977) Structure and thermodynamic properties of high density lipoprotein recombinants. J. Biol. Chem. **252:** 4701–4711
74 Sparks D. L., Phillips M. C. and Lund-Katz S. (1992) The conformation of apolipoprotein A-I in discoidal and spherical recombinant high density lipoprotein particles: 13C NMR studies of lysine ionization behavior. J. Biol. Chem. **267:** 25830–25838
75 Brasseur R., De Meutter J., Vanloo B., Goormaghtigh E., Ruysschaert J. M. and Rosseneu M. (1990) Mode of assembly of amphipathic helical segments in model high- density lipoproteins. Biochim. Biophys. Acta **1043:** 245–252
76 Wald J. H., Goormaghtigh E., De Meutter J., Ruysschaert J. M. and Jonas A. (1990) Investigation of the lipid domains and apolipoprotein orientation in reconstituted high density lipoproteins by fluorescence and IR methods. J. Biol. Chem. **265:** 20044–20050
77 Phillips J. C., Wriggers W., Li Z., Jonas A. and Schulten K. (1997) Predicting the structure of apolipoprotein A-I in reconstituted high-density lipoprotein disks. Biophys. J. **73:** 2337– 2346
78 Sheldahl C. and Harvey S. C. (1999) Molecular dynamics on a model for nascent high-density lipoprotein: role of salt bridges. Biophys. J. **76:** 1190–1198
79 Borhani D. W., Rogers D. P., Engler J. A. and Brouillette C. G. (1997) Crystal structure of truncated human apolipoprotein A-I suggests a lipid-bound conformation. Proc. Natl. Acad. Sci. USA **94:** 12291–12296
80 Rogers D. P., Roberts L. M., Lebowitz J., Datta G., Anantharamaiah G. M., Engler J. A. et al. (1998) The lipid-free structure of apolipoprotein A-I: effects of amino-terminal deletions. Biochemistry **37:** 11714–11725
81 Rogers D. P., Roberts L. M., Lebowitz J., Engler J. A. and Brouillette C. G. (1998) Structural analysis of apolipoprotein A-I: effects of amino- and carboxy-terminal deletions on the lipid-free structure. Biochemistry **37:** 945–955
82 Koppaka V., Silvestro L., Engler J. A., Brouillette C. G. and Axelsen P. H. (1999) The structure of human lipoprotein A-I: Evidence for the 'belt' model. J. Biol. Chem. **274:** 14541–14544
83 Axelsen P. H., Braddock W. D., Brockman H. L., Jones C. M., Dluhy R. A., Kaufman B. K. et al. (1995) Use of internal reflectance infrared spectroscopy for the in-situ study of supported lipid monolayers. Appl. Spectrosc **49:** 526–531
84 Silvestro L. and Axelsen P. H. (1999) FTIR linked analysis of conformational changes in annexin V upon membrane binding. Biochemistry **38:** 113–121
85 Wimley W. C., Hristova K., Ladokhin A. S., Silvestro L., Axelsen P. H. and White S. H. (1998) Folding of Beta-Sheet Membrane Proteins: A Hydrophobic Hexapeptide Model. J. Mol. Biol **277:** 1091–1110
86 Citra M. J. and Axelsen P. H. (1996) Determination of molecular order in supported lipid membranes by internal reflection Fourier transform infrared spectroscopy. Biophys. J. **71:** 1796–1805
87 Axelsen P. H. and Citra M. J. (1997) Orientational Order Determination by Internal Reflection Infrared Spectroscopy. Prog. Biophys. Mol. Biol. **66:** 227–253
88 Silvestro L. and Axelsen P. H. (1998) Infrared Spectroscopy of Supported Lipid Monolayer, Bilayer, and Multibilayer Membranes. Chem. Phys. Lipids **96:** 69–80

89 Segrest J. P., Jones M. K., Klon A. E., Sheldahl C. J., Hellinger M., De Loof H. et al. (1999) A detailed molecular belt model for apolipoprotein A-I in discoidal high density lipoprotein. J. of Biol. Chem. **274:** 31755–31758
90 Sirtori C. R., Calabresi L. and Franceschini G. (1999) Recombinant apolipoproteins for the treatment of vascular diseases. Atherosclerosis **142:** 29–40
91 Klon A. E., Jones M. K., Segrest J. P. and Harvey S. C. (2000) Molecular belt models for the apolipoprotein A-I Paris and Milano mutations. Biophys. J. **79:** 1679–1681
92 Calabresi L., Vecchio G., Frigerio F., Vavassori L., Sirtori C. R. and Franceschini G. (1997) Reconstituted high-density lipoproteins with a disulfide-linked apolipoprotein A-I dimer: evidence for restricted particle size heterogeneity. Biochemistry **36:** 12428–12433
93 Daum U., Langer C., Duverger N., Emmanuel F., Benoit P., Denefle P. et al. (1999) Apolipoprotein A-I (R151C) Paris is defective in activation of lecithin : cholesterol acyltransferase but not in initial lipid binding, formation of reconstituted lipoproteins, or promotion of cholesterol efflux. J. Mol. Med. **77:** 614–622
94 Maiorano J. N. and Davidson W. S. (2000) The orientation of helix 4 in apolipoprotein A-I-containing reconstituted high density lipoproteins. J. Biol. Chem. **275:** 17374–17380
95 Li H., Lyes D. S., Thomas M.J., Pan W. and Sorci-Thomas M. G. (in press) Structural determination of lipid-bound apo A-I using fluorescence resonance energy transfer. J. Biol. Chem.