[Frontiers in Bioscience 8, d1044-1054, May 1, 2003]

HIGH DENSITY LIPOPROTEIN STRUCTURE

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1. ABSTRACT

HDL particles possess important antiatherogenic functionalities and understanding of the molecular mechanisms underlying these effects requires detailed knowledge of HDL structure. This review summarizes current understanding of HDL structure. The various HDL subclasses are compared in terms of their lipid and protein compositions. The lipid-binding properties of the principal HDL apolipoprotein, apo A-I, permit plasticity in HDL structure. The amphipathic alpha-helical domains that are the major element of secondary structure mediate the interaction of apo A-I with phospholipid. Low resolution models of the structures of both discoidal and spherical HDL particles are evaluated. HDL particles are dynamic in that they are being remodeled constantly *in vivo* by interaction with lipases, lipid transfer proteins, and cell-surface HDL receptors. Current knowledge of the ways in which HDL particle structure is modulated by interactions with proteins such as LCAT, CETP, SR-BI and ABCA1 is reviewed.

2. INTRODUCTION

It is established that high density lipoprotein (HDL) subclasses exist in human plasma and that the various species behave differently with respect to important physiological functions such as the mediation of cellular cholesterol efflux (1). The factors that give rise to the structural heterogeneity of HDL are summarized in Figure 1. Since epidemiological studies have shown that there is an inverse correlation between the risk of coronary artery disease and plasma HDL cholesterol levels (2), there has been a lot of research activity directed at understanding the antiatherogenic properties of the various HDL subspecies and their principal apolipoprotein, apo A-I (3). The protective properties of HDL are generally thought to be related to the following functions of HDL: 1) mediation of the reverse transport of cholesterol from peripheral cells (including macrophage foam cells in atherosclerotic lesions) to the liver for excretion from the body, 2) limitation of oxidative modification of low density lipoprotein (LDL) due to the activity of HDL-associated enzymes such as paraoxonase and platelet-activating factor acetylhydrolase (4), and 3) attenuation of inflammation (5) .

To understand the mechanisms underlying the above effects, it is necessary to know the structures of the various HDL subspecies. This review summarizes current knowledge of human HDL structure. The aspects that are summarized include HDL particles composition, the structures of the major HDL subspecies and apo A-I, as well as the structural consequences of HDL remodeling by plasma proteins and cell surface receptors.

3. HDL COMPOSITION

Traditionally, HDL isolated by ultracentrifugation is defined as the lipoprotein with density in the range 1.063–1.21 g/ml (6). However, HDL constitutes a heterogeneous group of particles differing in density, size, electrophoretic mobility, lipid composition, and apolipoprotein content (Figure 1). Therefore, HDL can

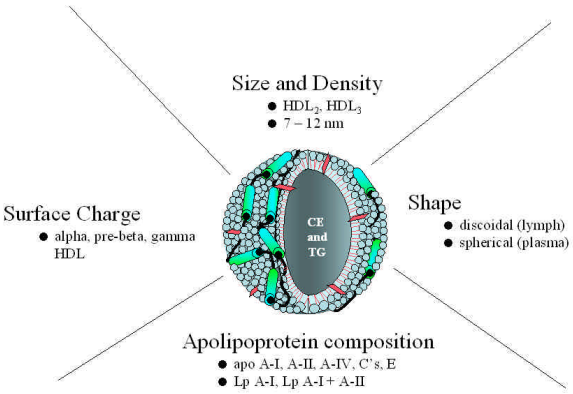

Figure 1. Factors contributing to the structural heterogeneity of HDL particles. Different subclasses of the depicted spherical HDL particle can be isolated on the basis of differences in particle diameter and density (lipid/protein ratio). Variations in the surface charge lead to subclasses that can be separated due to differences in electrophoretic mobility. Differences in apolipoprotein composition permit isolation of HDL particles containing either apo A-I alone (Lp A-I) or apo A-I plus apo A-II (Lp A-I + A-II) by immunoaffinity methods. HDL particles can also be distinguished by their shape: spherical plasma HDL particles contain a neutral lipid core whereas the discoidal HDL that occur in lymph do not. See text for further details.

be fractionated into discrete subclasses by different techniques according to their physicochemical properties. Early ultracentrifugal studies separated human HDL into two subfractions on the basis of density, HDL_2 (1.063-1.125 g/ml) and HDL_3 (1.125-1.21 g/ml) (7). HDL_2 and HDL_3 can be further divided into HDL_{2b}(10.6 nm), HDL_{2a}(9.2 nm), HDL_{3a}(8.4 nm), HDL_{3b}(8.0 nm), and HDL_{3c} (7.6 nm) in decreasing order of particle diameter on gradient gel electrophoresis (8,9). HDL can be separated into two main subpopulations on the basis of electrophoretic mobility; the major subfraction has the same mobility as alpha-globulin and is called alpha HDL, while the other migrates similarly to pre-beta globulin and is called pre-beta HDL (10,11). Most of the HDL particles in plasma are alpha HDL (12), and pre-beta HDL represents only 2-14% of total apo A-I (10,13,14). The density of pre-beta HDL is higher than 1.21 g/ml (15), so it is not included in HDL recovered from serum by ultracentrifugation in the traditional density interval of 1.063–1.21 g/ml and pre-beta HDL was not recognized until the 1980's. Pre-beta HDL has also been resolved into pre-$beta_1$, pre-$beta_2$, and pre-$beta_3$ HDL particles according to increasing size by two-dimensional electrophoresis using agarose gel in the first dimension and nondenaturing polyacrylamide gradient gel electrophoresis in the second dimension (11,16). HDL can also be separated on the basis of apolipoprotein composition into several subpopulations using immunoaffinity methods (17). Two major apo A-I-containing HDL particles exist, i.e. Lp A-I+A-II, which includes both apo A-I and apo A-II, and Lp A-I, which contains only apo A-I . Both Lp A-I+A-II and Lp A-I particles possess alpha electrophoretic mobilities and hydrated densities in the range of HDL (18). Other minor particles have been reported as well such as Lp A-I+A-IV containing apo A-I and apo A-IV, and Lp A-IV containing only apo A-IV (19). Of the total recovered weight of apo A-I in human plasma, 65% is found in Lp A-I+A-II, 25% in Lp A-I, and only 1-2% in Lp A-I+A-IV. Most of the apo A-II is found in Lp A-I+A-II, with a small proportion associated with apo A-IV (19). Table 1 summarizes the main characteristics of the major human HDL subclasses including pre-beta HDL, HDL_2 and HDL_3.

3.1. Pre-beta HDL

Pre-beta HDL contains mainly apo A-I and phospholipids (PL) with small amounts of cholesterol (10, Table 2). Triglyceride (TG) has been found in the pre-$beta_1$ HDL fraction isolated from human ovarian follicular fluid (20) but not in the fraction isolated from human plasma (10). Apo A-I is the predominant apolipoprotein in pre-beta HDL, while apo A-II, apo B, apo C-III and apo E are not detectable (10,11). In addition to apo A-I, pre-$beta_3$ HDL contains lecithin: cholesterol acyltransferase (LCAT), and cholesteryl ester transfer protein (CETP) (16). Lipid-free apoA-I and discoidal apo A-I /phosphatidylcholine (PC) complexes migrate with pre-beta mobility (15,21). In contrast to pre-beta HDL particles, alpha HDL particles are spherical in shape due to the presence of a neutral lipid (cholesteryl ester (CE) and TG) core in the particles (see Section 4.3); the presence of a core alters the conformation of apo A-I so that the net negative surface charge on the particle increases and alpha mobility ensues (21). Pre-$beta_1$ HDL is considered to be the first acceptor of cellular unesterified (free) cholesterol (FC) (11,22) and is therefore critical for reverse cholesterol transport (23). After cellular FC is initially taken up by pre-$beta_1$ HDL, it is thought to be transferred in the sequence pre-$beta_2$ HDL$\rightarrow$ pre-$beta_3$ HDL $\rightarrow$ alpha HDL (22). Another minor subfraction of HDL, gamma-LpE, has gamma electrophoretic mobility and apo E as its only apolipoprotein and it is rich in sphingomyelin (SM) (24). Gamma-LpE is probably another initial acceptor of cell-derived cholesterol similar to pre-$beta_1$ HDL (24).

3.2. Alpha HDL

Alpha HDL consists of approximately 50% protein, 25% PL, 20% cholesterol and 5% TG by weight (25). However, as described earlier, there are several subpopulations in alpha HDL. Table 2 lists the chemical compositions of the two major subclasses of alpha HDL, i.e. HDL_2 and HDL_3 (25). As shown in Table 2, there is more protein and less lipid in HDL_3 compared to HDL_2. The calculated lipid composition is 137 PL, 50 FC, 90 CE and 19 TG molecules in an HDL_2 particle, and 51 PL, 13 FC, 32 CE and 9.5 TG molecules in an HDL_3 particle (26). PC is the major PL and constitutes some 70-80% of the PL in HDL (12). Other PL include lysolecithin (3-11%), SM (12-14%), phosphatidylethanolamine and phosphatidylserine (4-9%), and phosphatidylinoositol (2%) (12). Thus, choline-containing PL (PC, lysolecithin, SM) represent 90-95% of the PL in HDL. In normal human HDL, five fatty acids are predominantly present in PC, i.e. palmitic (16:0, 29%), linoleic (18:2, 25%), stearic (18:0, 14%), oleic (18:1, 12%), and arachidonic (20:4, 10%) acids

Table 1. Characteristics of human HDL subclasses

Property	Pre-beta HDL	HDL_2	HDL_3
Molecular weight	Pre-$beta_1$: 71,000[11] Pre-$beta_2$: 325,000[11]	360,000[26,91]	175,000[26,91]
Electrophoretic mobility	Pre-beta[11]	alpha[12]	alpha[12]
Density (g/ml)	>1.210[92]	1.063-1.125[7]	1.125-1.210[7]
Subpopulations	Pre-$beta_1$, Pre-$beta_2$, Pre-$beta_3$[11] [16]	HDL_{2b}, HDL_{2a}[8,9]	HDL_{3a}, HDL_{3b}, HDL_{3c}[8,9]
Diameter (nm)	Pre-$beta_1$: 5.4-7[20,92] Pre-$beta_2$: 12-14[92]	8.8-12 HDL_{2b}: 9.7-12 HDL_{2a}: 8.8-9.7	7.2-8.8[9] HDL_{3a}: 8.2-8.8 HDL_{3b}: 7.8-8.2 HDL_{3c}: 7.2-7.8
Concentration in normal human plasma			
(mg apo A-I/dL)	6-8 [13,93,94]	13.9[95]	34.4[95]
(mg cholesterol/dL)		17.7[96,97]	30.6[96,97]

Superscripts represent reference numbers

Table 2. Chemical composition of human HDL subclasses

Chemical composition [1]	Pre-beta[10]	HDL_2[26,91,98,99]	HDL_3[26,91,98,99]
Apolipoproteins	91.3	40.2	55.5
Phospholipids	6.6	31.3	22.7
Free cholesterol	0.3	5.8	2.8
Cholesteryl esters	1.8	17.6	14.7
Triglyceride		4.2	3.4
Major apolipoproteins	apo A-I	apo A-I, apo A-II	apo A-I, apo A-II

Superscripts in paranthesis represent reference numbers, [1] Expressed as percentage of weight

(12). Regarding the proteins, apo A-I and apo A-II are the major HDL proteins comprising 70% and 20% of total HDL protein, respectively (19). Other minor apolipoproteins such as apo A-IV, apo Cs, and apo E are found in HDL (3). The molar ratio of apo A-I to apo A-II is approximately 2 to 1 in Lp A-I+A-II (18). Other HDL-associated proteins such as LCAT and CETP are more abundant in Lp A-I than in Lp A-I+A-II (27). It has been shown that the majority of Lp A-I has the same density and size as HDL_2, whereas the majority of Lp A-I+A-II floats with HDL_3 (18).

4. HDL AND APOLIPOPROTEIN STRUCTURE

Apo A-I is the principal apolipoprotein of human HDL (Table 2) and it plays a critical role in determining the structure of HDL particles. The mature human apo A-I molecule in plasma contains 243 amino acid residues in a single polypeptide chain which is about 55% and 75% alpha-helical in the lipid-free and lipid-associated states, respectively (28,29). Analysis of the primary structure of apo A-I has allowed the identification of 11- and 22-residue sequence repeats usually separated by proline-containing segments (30,31). There are thought to be eight 22-mer and two 11-mer tandem amino acid sequence repeats (32). Each of these repeats has the periodicity of an amphipathic alpha-helix and this structural motif mediates interaction of the protein with PL-water interfaces. The hydrophobic face of the helix inserts into the nonpolar lipid milieu while the hydrophilic face of the helix interacts with the aqueous phase (30). The amphipathic alpha-helices have been classified according to the distribution of charged residues in the hydrophilic face (30). Some of the functional domains in the human apo A-I molecule are indicated in Figure 2A and a noteworthy feature is that the C-terminal region is very hydrophobic, as might be expected for a lipid-binding domain. However, the hydrophobicity analysis of the amino acid sequence does not point to other known lipid-binding regions. It is necessary to take the secondary structure of apo A-I into consideration to identify residues 44-65 as a strongly lipid-binding region. Figure 2B shows a predicted distribution of amphipathic alpha-helices in the apo A-I molecule; the 22-mer peptides 44-65 and 220-241 have the highest lipid affinities (33).

Lipid-free apo A-I is known to self-associate in aqueous solution, a process which may stabilize the protein. However, at low concentration (<0.1 mg/ml) only the monomeric form is detected (34). The monomer is loosely folded and conformationally flexible (35) exhibiting the characteristics of a molten globular state (36). Sedimentation velocity experiments on human apo A-I reveal significant conformational heterogeneity supporting a conformational plasticity consistent with a molecular model that incorporates both a globular, helical bundle and an open rod-shaped conformation (Figure 3A) (37). It has been suggested that the overall shape of the lipid-free apo A-I monomer in solution is an elongated ellipsoid (Figure 3A) with an axial ratio ~ 5/1 and dimensions ~2.5 x 12.5nm (38).

4.1. Interaction of apo A-I with lipid

The flexible molten globular-like state for lipid-free apo A-I explains its lipid-binding properties (35). The loose arrangement of the alpha-helices allows rapid interaction of exposed hydrophobic regions of the protein with lipid. It is well established that isolated apo A-I can interact spontaneously with dimyristoyl

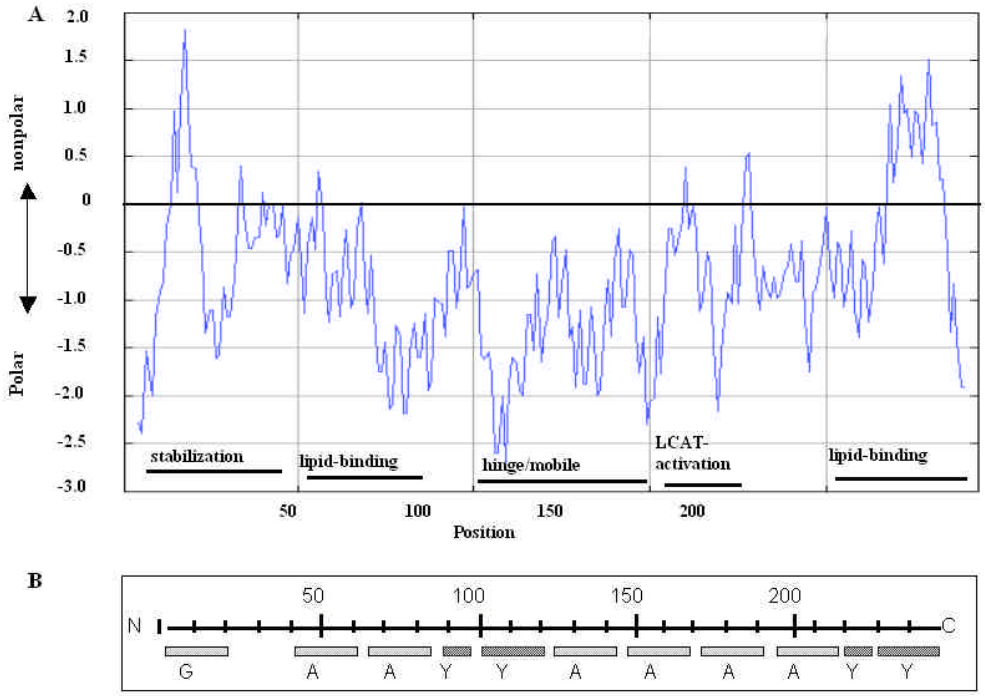

Figure 2. Analysis of the amino acid sequence of human apo A-I. Panel A. Hydropathy plot (Kyte-Doolittle scale) showing variations in polarity along the 243 amino acid sequence. The functionalities that have been suggested for various regions of the apo A-I molecule are indicated by the horizontal lines at the bottom of the plot. The N-terminal domain is thought to stabilize the protein conformation and the central hinge/mobile region is thought to move on and off HDL particle surfaces as the particle size changes. See text for details. Panel B. The predicted 11-mer and 22-mer amphipathic alpha-helices with the class of each helix shown (30).

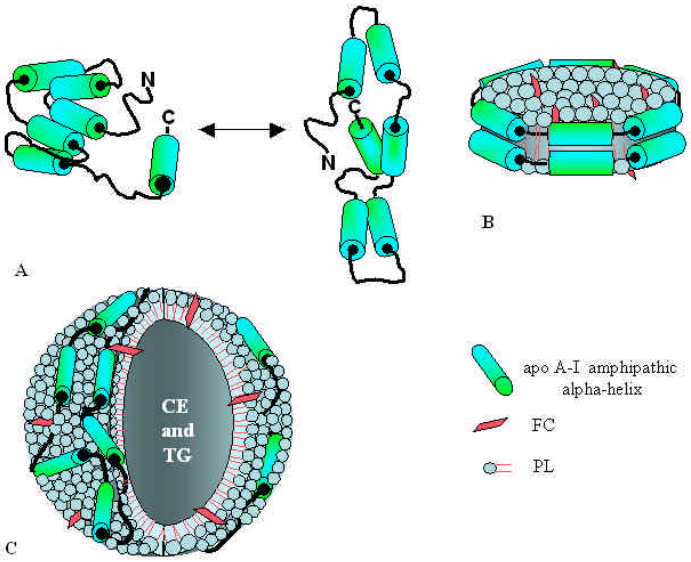

Figure 3. Proposed molecular models for lipid-free and lipid-associated apo A-I. Panel A. Model of lipid-free apo A-I incorporating both a globular, helical-bundle structure and an open rod-shaped conformation (43,44). Panel B. Molecular belt model for a discoidal POPC/apo A-I complex. The organization of the amphipathic alpha-helices are perpendicular to the direction of the PL acyl chains. The pair of apo A-I molecules are oriented antiparallel to each other allowing for a perfect alignment of interhelical salt-bridge pairs between them (49-52). Panel C. The "oil-drop" model of spherical HDL in which the lipids and apolipoproteins are held together largely by noncovalent forces. The structure is an approximately 10 nm-diameter sphere with a nonpolar core of CE and TG molecules encapsulated by a surface monolayer of amphipathic alpha-helical apolipoprotein and PL molecules.

phosphatidylcholine (DMPC) at its gel-liquid crystal phase transition temperature (24°C) and form discoidal complexes (39). Similarly, removal of sodium cholate by dialysis from a ternary complex of apo A-I/egg PC/sodium cholate results in the formation of discoidal lipoproteins (40,41). The C-terminal domain of apo A-I is critical in determining both the rate and degree of binding to lipids (42,43). The C-terminal 50 residues are thought to become more alpha-helical upon lipid-association (44) although the extent of this effect is in question because stable helices exist in the C-terminal domain (45). Consistent with a key role for the C-terminal, an amphipathic alpha-helical peptide comprising residues 220-241 of apo A-I has high lipid-binding affinity and, unlike most of the other alpha-helical domains in apo A-I, is able to spontaneously form discoidal complexes when incubated with DMPC (33). However, the N-terminal residues 44-65 also exhibit strong lipid-binding characteristics (33) and, together with some central domain helices (46), they probably modulate how the apo A-I molecule binds to lipid.

4.2. Discoidal HDL

The most studied and best-characterized type of HDL particle is the discoidal complex comprising a small segment of PC bilayer surrounded at the edge by apo A-I molecules (Figure 3B). Two general models have been proposed for the orientation of apo A-I molecules at the edge of such a discoidal complex which typically contains some 200 PL molecules and 2 apo A-I molecules. These are the "picket fence" model where the 22-mer amphipathic alpha-helical repeats of apo A-I form tandem antiparallel helixes that are parallel to the lipid acyl chains and the "belt" model where molecules of apo A-I form an essentially continuous series of amphipathic alpha-helices that are aligned perpendicular to the direction of the PL acyl chains.

A detailed molecular model of the "picket-fence" arrangement for discoidal HDL was designed to match the known physical properties of a complex containing two molecules of apo A-I (47). In this model, eight 22-mer helical repeats span the circumference of the disc producing an average distance of about 15 Å between antiparallel alpha-helices. On the other hand, the N-terminal truncated apo A-I molecule missing residues 1-43 has been crystallized (48) and its secondary structure does not contain antiparallel alpha-helices but rather comprises a linear series of amphipathic alpha-helices punctuated by kinks at the regularly spaced proline residues. This conformation is thought to be similar to that in a lipid-associated state and has been utilized to generate models for the organization of apo A-I in a "belt-like" arrangement around the edge of discoidal complexes with PL (35) (Figure 3B). Recent fluorescence spectroscopic evidence has confirmed that the organization of most of the apo A-I helices are perpendicular to the direction of the PL acyl chains (49). Additional evidence in support of the "belt" conformation has been provided by application of polarized attenuated total internal reflection Fourier transform infrared spectroscopy (50). In a "belt" model of a complex consisting of two antiparallel apo A-I molecules, there is a perfect alignment of the 20 interhelix salt-bridge pairs (51). The model, therefore, preserves the nearly perfect correspondence of basic and acidic residues observed in the crystal structure of the N-terminal truncated apo A-I missing residues 1-43 (48) so that salt-bridges can form between a pair of apo A-I molecules oriented antiparallel to each other. In addition to the molecular "belt" model for apo A-I bound to discoidal particles, a helical "hairpin" model for apo A-I in discoidal reconstituted HDL particles is also proposed (44,48,52). The helical "hairpin" model offers an explanation of the discrete particle size heterogeneity observed in reconstituted HDL particles; in particular, it provides a model for discoidal reconstituted HDL particles containing three apo A-I molecules. Two different models for organization of apo A-I on such discoidal particles have been proposed (52), and in both models, the helical "hairpin" plays a critical structural role. In one model, two monomers of apo A-I associate in an antiparallel fashion, while a third monomer is present as a helical "hairpin". The second model proposes that the three monomeric apo A-I molecules in the helical "hairpin" conformation interact with each other by either a head-to-head orientation or a head-to-tail orientation. The belt model (Figure 3B) probably also applies to discoidal apo A-II/PL complexes because the crystal structure of an apo A-II/beta-octyl glucoside complex indicates that amphipathic alpha-helices are oriented perpendicular to the acyl chains (53).

4.3. Spherical HDL

Pre-beta and discoidal HDL particles are converted to mature, alpha-migrating, spheroidal HDL particles by the action of LCAT (16) and are remodeled further by the action of plasma enzymes and transfer proteins (see Section 5.2). The structure of spheroidal lipoprotein particles can be described by an "oil drop" model in which the lipids and the apolipoproteins are held together largely by noncovalent forces. On the basis of compositional and space- and surface-filling requirements, structural models of spherical HDL particles have been proposed. The generally accepted structure for a human HDL particle is an approximately 10 nm-diameter sphere which features a surface layer of protein and amphiphilic lipid molecules encapsulating a nonpolar core of CE and TG molecules (Figure 3C). The PL, CE, and TG (Table 2) in such HDL particles are in a disordered, fluid state. NMR experiments indicate that the FC molecules are present in two distinct environments (54). The cholesterol molecules dissolved in the core are disordered and mobile while the cholesterol molecules in the surface of the particle undergo relatively restricted, anisotropic motions. The cholesterol molecules in the two locations undergo fast exchange on the biological time scale, indicating that >90% of the FC molecules in HDL are in a single kinetic pool for exchange with FC molecules in other lipoprotein particles or cells. The spheroidal HDL particles are quite heterogeneous and differ in the relative proportions of lipid classes, in the lipid to protein ratio and in the apolipoprotein species present, resulting in differences in size, density and electrophoretic mobility (See Tables 1 and 2). Spherical HDL particles contain several apolipoprotein molecules but the organization of the alpha-helices in apo A-I molecules on spherical HDL particles is not known. In addition, PL-

Table 3. Factors involved in remodeling of HDL particles

Factors	Effect on HDL particle size		Mechanism
	Increase	decrease	
ABCA1	+		Lipidation of apolipoproteins
LCAT	+		Formation of CE core, fusion
PLTP	+		Fusion of small HDL particles
CETP		+	Transfer of CE
HL		+	Removal of surface lipids
SR-BI		+	Selective uptake of lipids into cells

apolipoprotein interactions, as they occur on the surface of HDL particles, are not understood fully. The presence of apolipoprotein at a PL-water interface does not perturb the PC polar group conformation which is determined by intramolecular effects (55). The presence of apo A-I molecules also has little effect on the dynamics of the lipid molecules. As far as PL conformation is concerned, the primary effect of amphipathic alpha-helix-forming apolipoproteins at the surface of lipoprotein particles is spacing out of the PL molecules. The finding that the insertion of apo A-I does not significantly change the polar group conformation of the lipid suggests that electrostatic forces do not play a major role in the lipid-protein interaction. Rather, it points to hydrophobic interactions being the dominant stabilizing force. In a recent study of the binding of apo E to a homogeneous emulsion particle (56), it was demonstrated that there are two lipid-bound conformations of apo E. The N-terminal 4-helix bundle can adopt either an open or a closed conformation, depending upon the surface concentration of emulsion-bound apo E. At high surface concentration, the helix bundle is closed and not in contact with the emulsion particle surface whereas, at low surface concentration, all alpha-helices in the apo E molecule are in contact with the surface. Since apo A-I and apo E are in the same gene family and the N-terminal helices in apo A-I also form a bundle (57), it is reasonable to postulate that apo A-I interacts similarly with spherical lipoprotein particles. Thus, at low surface concentration, most alpha-helices will be in contact with the PL-water interface; a decrease in particle size that raises the apolipoprotein surface concentration forces some helices out of contact with the particle surface. By analogy to the apo E situation, these helices may be part of a helix bundle located towards the N-terminal of the apo A-I molecule. Indeed, a pair of relatively polar alpha-helices near residue 100 are thought to form a "hinge domain" (58) (Figure 2) that allows the apo A-I molecule to adapt to the surface of HDL particles of different diameters. Major changes in HDL particle size due to remodeling lead to movement of entire apo A-I molecules on and off the surface. Dissociation of apo A-I molecules in this fashion leads to creation of a pool of "lipid-poor" pre-beta HDL (59).

5. MODIFICATION OF HDL STRUCTURE *IN VIVO*

The sites of HDL synthesis and the factors that contribute to the formation of the particles *in vivo* are a subject of intense investigation. As discussed above, HDL exists as a heterogeneous population of particles varying in size, hydrated density, and lipid and protein components. Many factors modify HDL structure and these changes can be induced by both plasma factors such as lipid transfer proteins and lipases, as well as by cell surface HDL or apolipoprotein receptors (Table 3).

5.1. Biogenesis of HDL

The initial step in the formation of HDL is the acquisition of lipid by apo A-I, the major apolipoprotein associated with HDL which is secreted by the liver and intestine (Figure 4). Although the exact mechanism remains elusive, the lipidation of apo A-I can occur extracellularly (60,61) or the apolipoprotein can be secreted as a nascent lipidated particle (62-64). The structure of these initial HDL particles has not been well defined but secreted particles that are either spherical (63) or discoidal (64) in shape have been observed. Recently, studies of patients with Tangier disease, who have almost no detectable circulating HDL, identified the ATP-binding cassette (ABC)-A1 transporter as the mutated gene that is responsible for the phenotype (65,66). This transmembrane glycoprotein mediates efflux of cellular PL and FC to lipid-free or lipid-poor apo A-I (67) in the extracellular medium to form nascent HDL particles.

5.2. Maturation and remodeling of HDL in the plasma compartment

Through an ABCA1-mediated process, apo A-I eventually acquires sufficient PL and FC to form a nascent HDL particle consisting of a segment of PL bilayer with apo A-I molecules around the circumference stabilizing it (Figure 3B). This nascent HDL particle then undergoes a series of enzymatically-driven modifications that results in the formation of a spherical HDL particle. The first of the steps in the conversion of discoidal HDL to a small spherical HDL particle occurs through the action of LCAT (16,68). This plasma glycoprotein of 49 kDa is secreted mainly by the liver and adipose tissue and its association with HDL is influenced by the lipid and not the apolipoprotein component of the lipoprotein (69). LCAT catalyzes the transesterification of an sn-2 fatty acid from PC, which is the most abundant PL in HDL, to the 3-hydroxyl group of cholesterol forming CE (68). The reaction requires apo A-I on the HDL surface as a cofactor (70). The newly formed CE molecules move into the center of the discoidal HDL particle and create a hydrophobic core, thereby making the particle spherical in shape. These small spherical particles which are ~ 8 nm in diameter contain on average, two apo A-I molecules (16) embedded in a surface monolayer of PL molecules (Figure 3C).

Through the continued activity of LCAT and another plasma protein, phospholipid transfer protein (PLTP), these small spherical HDL particles become the

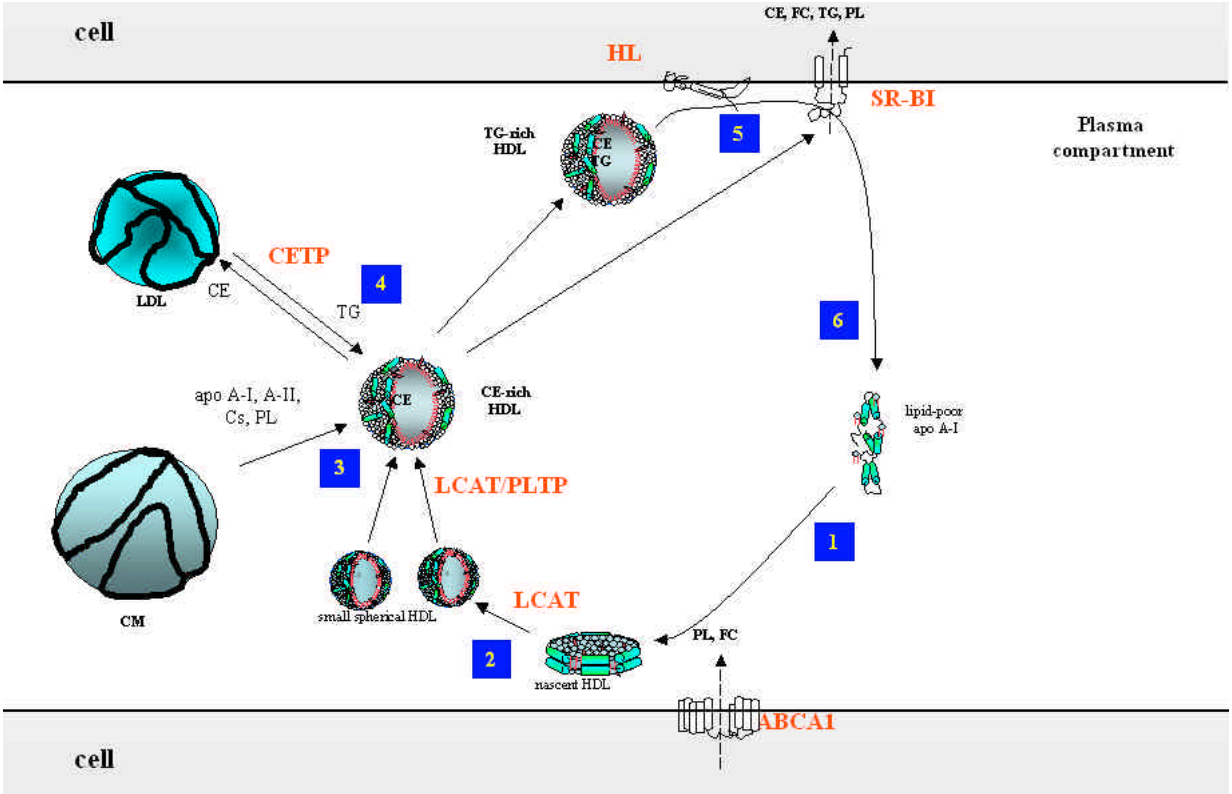

Figure 4. Remodeling of HDL in the plasma compartment: 1) Secreted lipid-free or lipid-poor apo A-I from hepatocytes and enterocytes picks up PL and FC from cell plasma membrane via ABCA1. Nascent HDL and small spherical HDL can also be secreted by hepatocytes and enterocytes (see text). 2) LCAT esterifies the FC to CE converting the nascent HDL to small spherical particles. 3) LCAT/PLTP form large spherical HDL particles by mediating the fusion of small particles; these particles acquire additional apo A-I, apo A-II, apo Cs and PL from chylomicron (CM) remnants. 4) CETP exchanges TG and CE between apo B-containing lipoproteins (LDL) and CE-rich HDL to create TG-rich HDL. 5) HL hydrolyzes some of the HDL lipids and in cells expressing SR-BI, the HDL lipids (particularly CE and FC) are selectively taken up by cells. 6) The combination of HL and CETP activity creates a pool of lipid-free (poor) apo A-I. This pool of apo A-I can pick up additional lipids to form nascent HDL thereby recirculating in the plasma or some of it can be removed from the circulation by filtration in the kidneys.

mature spherical HDL found in the circulation which contain three and sometimes four apo A-I molecules (71,72). The 54 kDa PLTP is a non-specific lipid transfer protein that can also mediate the transfer of PL to other classes of lipoproteins in addition to HDL. Enlargement of HDL particles is thought to involve the fusion of small HDL particles through the action of either LCAT (73) or PLTP (71) which creates an unstable fusion product that reorganizes into a large spherical HDL with concurrent removal of lipid-poor apo A-I. Another possible way for the formation of large HDL is through the acquisition of lipid-free or lipid-poor apo A-I by the small spherical HDL. As the HDL particles become larger and enriched in CE, some of the CE molecules are transferred to apo B-containing lipoproteins (e.g. very low-density lipoprotein, chylomicrons) in exchange for TG. This enrichment of HDL with TG is mediated by CETP (74). The action of CETP is complemented by that of hepatic lipase (HL), an enzyme secreted mainly by the liver as well as adipocytes. This lipolytic enzyme is located on the membranes of endothelial cells lining the capillaries of the liver and hydrolyzes HDL TG and PL (75). The TG-rich HDL particles that results from the action of CETP are the preferred substrate of HL (76). Similar to the action of CETP, HL can catalyze the formation of pre-beta HDL from a large spherical TG-rich HDL (77). As the size of the HDL is decreased by HL, the surface area of the particle is reduced resulting in the shedding of apo A-I molecules. Thus, contrary to the actions of LCAT and PLTP, CETP and HL generate small HDL particles resembling pre-beta HDL (78).

Plasma HDL consists of particles predominantly containing either apo A-I only or a mixture of apo A-I and apo A-II (Table 2). Much like apo A-I, apo A-II is secreted as a lipid-poor protein by the liver (79) and is the second most abundant apolipoprotein on HDL. The mechanism by which apo A-II becomes associated with HDL is not known. Similar to apo A-I, apo A-II is able to remove PL and FC from cells to form discoidal HDL particles, but since apo A-II cannot activate LCAT, these particles do not mature into spherical HDL (80). Therefore, the transfer of apo A-II from chylomicrons or fusion of apo A-II-only HDL particles with apo A-I-containing HDL are most likely the pathways by which apo A-II becomes incorporated into mature HDL (78). In addition to apo A-II,

other apolipoproteins such as apo Cs and apo E may be incorporated into apo A-I-containing HDL by movement out of other classes of lipoproteins.

5.3. Catabolism of HDL

A good portion of the CE in HDL is the precursor for steroid hormone synthesis in the steroidogenic tissue (81). In contrast to the receptor-mediated endocytosis of LDL by the LDL receptor, CE from HDL is taken into cells selectively without whole particle uptake (82). This selective CE uptake pathway is mediated by the scavenger receptor (SR)-BI (83) which is expressed by a wide variety of tissues that undergo selective CE uptake (84). SR-BI has higher affinity for lipid-rich HDL than lipid-poor HDL (85). Hence, mechanistically, as the CE- and TG-rich HDL bind to SR-BI, CE, TG, FC and some PL molecules preferentially diffuse out of the HDL particle into the cell plasma membrane (86) down their concentration gradient. This process is significant for the catabolism of HDL lipids (87,88). The reduction in HDL particle size associated with the loss of lipid molecules promotes removal of apolipoprotein molecules. HDL apolipoproteins are removed from the circulation when they are in lipid-free (poor) state by filtration in the kidneys (89,90).

6. CONCLUSIONS AND PERSPECTIVES

HDL structure is complex because of the existence of subclasses of particles and because of the remodeling that is induced by interaction with lipases, lipid transfer proteins and HDL receptors on cell surfaces. The principal apolipoprotein of HDL, apo A-I, can support this structural plasticity because it is a conformationally flexible molecule that can bind to lipoprotein particle of different sizes. The key structural motif in the apo A-I molecule that mediates lipid binding is the amphipathic alpha-helix. X-ray and NMR methods have not yet been applied successfully to solve the structure of an HDL particle at the level of atomic resolution. The absence of atomic resolution definition of the structures of HDL particles has hampered the elucidation of the molecular mechanisms by which the different types of HDL particles achieve their biological functions. Nonetheless, the low resolution structural models of the HDL subclasses (Figure 3) are reasonably well established and are proving helpful for understanding the antiatherogenic properties of HDL. Importantly, the structure-function relationships of apo A-I are being dissected successfully using protein engineering techniques. It can be anticipated that the mechanisms by which this protein acts as a cofactor for LCAT, and a ligand for the receptors SR-BI and ABCA1 will be uncovered in the near future. Another aspect of HDL structure that deserves attention is the role of apo A-II because its contribution to HDL function is quite different to that of apo A-I.

7. ACKNOWLEDGEMENTS

The work in this laboratory was supported by NIH grants HL22633 and HL 56083.

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Abbreviations: ABCA1, ATP-binding Cassette Transporter A1; apo, apolipoprotein; CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; DMPC, dimyristoyl phosphatidylcholine; FC, free (unesterified) cholesterol; HDL, high density lipoprotein; HL, hepatic lipase; LCAT, lecithin: cholesterol acyltransferase; LDL, low density lipoprotein; PC, phosphatidylcholine; PL, phospholipid; PLTP, phospholipid transfer protein; POPC, palmitoyl,oleoyl phosphatidylcholine; SR-BI, scavenger receptor B-I; TG, triglyceride

Key Words: HDL, Discoidal HDL, Lipoproteins, Apo A-I, Reverse cholesterol transport, Cholesterol, Alpha HDL, Pre-beta HDL, Amphipathic alpha-helix, Lipid composition, ATP-binding cassette transporter A1, Lecithin: cholesterol acyltransferase, Scavenger receptor class B type I, Phospholipid transfer protein, Review

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