

Correlation of Structural Stability with Functional Remodeling of High-Density Lipoproteins: The Importance of Being Disordered[†]

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ABSTRACT: High-density lipoproteins (HDLs) are protein–lipid assemblies that remove excess cell cholesterol and prevent atherosclerosis. HDLs are stabilized by kinetic barriers that decelerate protein dissociation and lipoprotein fusion. We propose that similar barriers modulate metabolic remodeling of plasma HDLs; hence, changes in particle composition that destabilize HDLs and accelerate their denaturation may accelerate their metabolic remodeling. To test this notion, we correlate existing reports on HDL-mediated cell cholesterol efflux and esterification, which are obligatory early steps in cholesterol removal, with our kinetic studies of HDL stability. The results support our hypothesis and show that factors accelerating cholesterol efflux and esterification in model discoidal lipoproteins (including reduced protein size, reduced fatty acyl chain length, and/or increased level of cis unsaturation) destabilize lipoproteins and accelerate their fusion and apolipoprotein dissociation. Oxidation studies of plasma spherical HDLs show a similar trend: mild oxidation by Cu²⁺ or OCl[−] accelerates cell cholesterol efflux, protein dissociation, and HDL fusion, while extensive oxidation inhibits these reactions. Consequently, moderate destabilization may be beneficial for HDL functions by facilitating insertion of cholesterol and lipophilic enzymes, promoting dissociation of lipid-poor apolipoproteins, which are primary acceptors of cell cholesterol, and thereby accelerating HDL metabolism. Therefore, HDL stability must be delicately balanced to maintain the structural integrity of the lipoprotein assembly and ensure structural specificity necessary for interactions of HDL with its metabolic partners, while facilitating rapid HDL remodeling and turnover at key junctures of cholesterol transport. The inverse correlation between HDL stability and remodeling illustrates the functional importance of structural disorder in macromolecular assemblies stabilized by kinetic barriers.

High-density lipoproteins (HDLs)¹ are heterogeneous complexes 7–13 nm in diameter that are composed of several major proteins (termed apolipoproteins), many minor proteins, and variable amounts of lipid molecules. Plasma concentrations of HDL and its major protein, apoA-I (243 amino acids), correlate inversely with the risk of developing coronary artery disease (reviewed in refs 1 and 2). This cardioprotection results from the central role of HDLs in reverse cholesterol transport (RCT) and from their antioxidant and anti-inflammatory action (3). RCT is the sole pathway of removal of cholesterol from peripheral tissues to the liver for excretion or to steroidogenic organs for hormone synthesis (1). During RCT, cell cholesterol is taken

up by nascent discoidal HDLs or their lipid-poor precursors (4), esterified by lecithin:cholesterol acyltransferase (LCAT), and sequestered in the core of HDLs, thereby converting them to mature spherical particles (Figure 1, steps 1–3). After further remodeling (steps 4 and 5), large spherical HDLs deliver their cargo of cholesterol esters to the liver via the selective lipid uptake mediated by the scavenger receptor BI (SR-BI, step 6) (5). Throughout RCT, HDLs undergo dynamic remodeling by lipophilic enzymes, lipid transfer proteins [such as cholesterol ester transfer protein (CETP)], and lipoprotein receptors (6). This may involve insertion of lipids (such as cholesterol) and proteins (such as LCAT and CETP), as well as apolipoprotein dissociation and HDL fusion that compensate for the imbalance between the particle core and surface upon remodeling. We propose that such remodeling is facilitated, in part, by the structural disorder in the HDL surface.

To test this notion, we correlate existing functional data, including studies of cell cholesterol efflux and esterification using reconstituted or plasma HDLs of various compositions (7–13), with our kinetic stability studies of these lipoproteins (14–17) (Table S1 of the Supporting Information). Earlier studies, which were based on a thermodynamic approach to

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¹ Abbreviations: HDL, high-density lipoprotein; apo, apolipoprotein; RCT, reverse cholesterol transport; LCAT, lecithin:cholesterol acyltransferase; ABC, ATP-binding cassette transporter; SR-BI, scavenger receptor BI; CETP, cholesterol ester transfer protein; PLA₂, phospholipase A₂; PC, phosphatidylcholine.

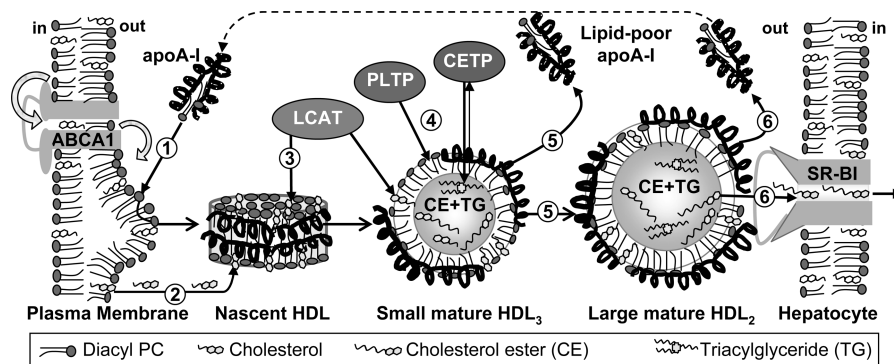


FIGURE 1: Simplified diagram of reverse cholesterol transport. (1) Lipid-poor apoA-I interacts with protrusions on the cell surface created by the ABCA1 transporter. (2) This active lipid transport leads to formation of nascent discoidal HDLs that accept additional cell cholesterol via such mechanisms as passive diffusion or interactions with the ABCG1 transporter. (3) LCAT action leads to accumulation of cholesterol ester (CE) in the core of nascent discoidal HDLs, converting them into mature spherical particles. (4) These small HDL₃ are further remodeled by LCAT and other plasma factors, such as phospholipid and cholesterol ester transfer proteins (PLTP and CETP, respectively). (5) The resulting imbalance between the particle core and surface is compensated by HDL fusion and dissociation of lipid-poor apoA-I. (6) Interactions of HDL and hepatic scavenger receptor (SR-BI) lead to selective uptake of apolar core lipids and HDL disintegration; the dissociated apoA-I re-enters RCT as lipid-poor or lipid-bound species.

HDL stability such as spectroscopic “end-point” measurements after incubation with denaturants (8–12 and references cited therein), revealed a correlation between HDL functions and lipid fluidity (7–9, 11) but showed no clear linkage with HDL stability. In contrast, our denaturation rate measurements in kinetic experiments (illustrated in Figure 1S of the Supporting Information) suggest an inverse correlation between HDL stability and metabolic remodeling in RCT (16, 17).

The kinetic approach to lipoprotein stability stems from the observation that HDL denaturation is a thermodynamically irreversible transition involving apolipoprotein unfolding, dissociation, and fusion of HDLs into larger particles that retain lipoprotein morphology (18–20). Upon further protein dissociation, spherical HDLs disintegrate, rupture, and release their apolar core lipids that coalesce into large droplets (19, 20). The slow unfolding rate, $k(T)$, of HDL proteins reflects a high free energy barrier, $\Delta G^* \propto -RT \ln k(T)$, that arises from transient disruption of protein and lipid interactions and transient solvent exposure of the apolar groups during protein dissociation and lipoprotein fusion and rupture. Unlike in the high-energy transition state, in native and fused HDL states most apolar groups are sequestered and polar groups are solvent-exposed, leading to the relatively low free energies of these states (Figure 2A). Kinetic lipoprotein stability is defined by the free energy barriers separating native from fused and denatured states.

Since both denaturation and metabolic remodeling of HDLs involve apolipoprotein dissociation and lipoprotein fusion, we proposed that these transitions are modulated by similar kinetic barriers (19, 20). Furthermore, since HDL rupture, which involves lipoprotein disintegration and release of the apolar lipids, occurs both at the final stage of denaturation and at the final stage of RCT during SR-BI-mediated lipid uptake (5, 21), these *in vitro* and *in vivo* transitions may involve similar kinetic barriers. If our hypothesis is correct, then less stable HDLs will tend to undergo faster metabolic remodeling during RCT. To test this notion, we correlate functional and kinetic stability studies of HDLs varying in protein and lipid composition. Below, we briefly describe the key steps in RCT and outline the role of structural disorder in HDL remodeling during

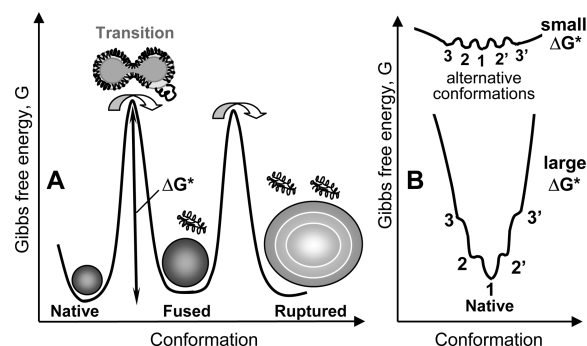


FIGURE 2: Free energy diagram illustrating global kinetic stability and local structural disorder. (A) The kinetic stability of native HDL (discoidal or spherical) is defined by the free energy barrier ($\Delta G^* = G_{\text{trans}} - G_{\text{native}}$) that decelerates protein dissociation and lipoprotein fusion (shown with a double arrow); G_{trans} refers to the high-energy transition state (16, 21). In spherical HDLs, the second kinetic step involves further protein dissociation, particle rupture, and release of apolar core lipids (19, 20). The order of magnitude of these barriers is ~ 20 kcal/mol (16, 18). (B) Linkage between the global structural stability and local disorder shown by zooming in on the free energy profile of the native state. If ΔG^* is small (low global stability, top), then the alternative conformations in the native state (marked 1–3) have comparable occupancies since they have similar free energies ($G_1 \cong G_2 \cong G_3 \dots$) and are separated by low energy barriers (comparable to the free energy of thermal motion, $RT \sim 0.6$ kcal/mol). If ΔG^* is large (high global stability, bottom), then $G_1 < G_2 < G_3$; hence, only state 1 is occupied with high probability, while states 2, 3, 4, etc., have significantly lower occupancies. Thus, low global stability facilitates structural flexibility (top), while high global stability hampers it (bottom).

these steps (summarized in Table S1 of the Supporting Information).

STRUCTURAL DISORDER FACILITATES EFFLUX OF LIPIDS FROM THE PLASMA MEMBRANE TO APOLIPOPROTEINS

ApoA-I and other exchangeable (water-soluble) apolipoproteins are secreted from the liver and gut in a lipid-free or lipid-poor form; lipid-poor apolipoproteins are also formed upon metabolic remodeling of HDLs and other lipoproteins (4, 21). These physiologically important species are rapidly converted to the lipid-bound state by binding to the existing lipoproteins or by taking up cell cholesterol and phospho-

lipids from plasma membranes. This active lipid efflux is mediated by ATP-binding cassette (ABC) transporter A1 (ABCA1), a transmembrane protein that moves lipids from the inner to the outer leaflet, thereby bending the membrane and creating protrusions that facilitate apolipoprotein insertion (22, 23) (Figure 1, step 1). Lipid-poor apolipoproteins, which are thought to comprise one protein and several lipid molecules, are the primary lipid acceptors in this process (4, 21). The structural stability of these transient species is probably comparable to the low thermodynamic stability of lipid-free apolipoproteins under nearly physiologic conditions ($\Delta G = G_{\text{unfolded}} - G_{\text{native}} < 2.5$ kcal/mol). This and other properties of apolipoproteins in solution (such as a compact globular shape with substantial secondary but loosely folded tertiary structure, low-cooperativity unfolding with a low effective enthalpy and low heat capacity increment, high aggregating propensity, binding of small apolar ligands, etc.) are characteristic of the molten globular state (24). Tertiary structure flexibility may reflect the lack of polar or charged residues on the large apolar faces of the apolipoprotein α -helices (25); hence, these helices cannot form buried H-bonds or salt bridges that confer specificity and stability to the structures of globular proteins. This tertiary structural plasticity may facilitate conformational changes necessary for apolipoprotein binding to the lipid surface (24, 26). In summary, structural disorder in the lipid acceptor (apolipoproteins) and the donor (plasma membrane distorted by ABCA1) facilitates the first step in RCT.

LOCAL DISORDER IN NASCENT DISCOIDAL HDLS FACILITATES CHOLESTEROL INSERTION AND ESTERIFICATION

Efflux of phospholipids and cholesterol to lipid-poor or lipid-free apolipoproteins results in the formation of nascent discoidal HDLs. These particles are comprised of the cholesterol-containing phospholipid bilayer and apolipoproteins wrapped around the disk perimeter in a belt-like α -helical conformation (27, 28). Nascent HDLs acquire additional cell cholesterol via passive diffusion (Figure 1, step 2) or active transport via ABCG1 (reviewed in ref 29) and form preferred substrates for LCAT.

What structural and stability properties of nascent HDLs facilitate cholesterol insertion and esterification? This was explored by using reconstituted discoidal HDLs with controlled composition, which provide excellent models for nascent HDLs. Davidson and colleagues studied binary complexes of apoA-I with diacyl PCs containing 14–18-carbon chains that were fully saturated or contained one or two cis double bonds (oleic or linoleic) in the *sn*-2 position; the results revealed that shorter-chain and cis-unsaturated PCs form more efficient acceptor particles for cell cholesterol (11). Our kinetic stability studies of apolipoprotein complexes with these lipids showed that shorter-chain and cis-unsaturated PCs form less stable disks: thus, a decrease in length by two CH₂ groups in fully saturated acyl chains or the presence of a cis-double bonds lowers the kinetic stability of apoC-I/PC disks by ≈ 1.4 kcal/mol [$\delta\Delta G^*(37^\circ\text{C})$] (16). A similar trend is observed in apoA-I/PC disks (ref 16 and Figures 2S and 3 of the Supporting Information). This suggests an inverse correlation between the kinetic stability of discoidal HDLs and their ability to accept cholesterol.

Studies of lipoproteins varying in protein composition further support this notion: binary complexes of DMPC with smaller and less helical proteins tend to be less stable (14, 15) and form more efficient cholesterol acceptors (12). Since the cells used in the functional studies contained little ABCA1 or ABCG1 (10, 11), cell cholesterol efflux in these studies occurred mainly via passive diffusion to discoidal HDLs. Thus, the inverse correlation between the stability of discoidal HDLs and their ability to accept cholesterol suggests that global destabilization of a lipoprotein increases local disorder in its surface (Figure 2B), thereby accelerating insertion of cholesterol into this surface.

Disorder in the lipid bilayer may also be important for PC lipolysis by the surface-active proteins, LCAT and phospholipase A₂ (PLA₂), whose function requires their insertion into the lipid surface. Hydrophobic packing defects in the lipid bilayer play a key role in insertion and activation of PLA₂ (30, 31). Since the PLA₂ reaction is the first step in the LCAT reaction (which hydrolyzes the *sn*-2 acyl chain from PC prior to transferring it to cholesterol to form cholesterol ester), LCAT activity is also expected to be enhanced by disorder in the HDL surface. Our results, together with studies of Pownall et al., support this notion: a decrease in acyl chain length from 18 to 12 carbons or an increase in the number of cis-double bonds from zero to one or two in diacyl PC complexes with apoA-I or apoC-I destabilizes these complexes (ref 16 and Figures S2 and S3 of the Supporting Information) and augments their ability to activate LCAT (7).

This differs from the effect of bilayer thickness on integral membrane proteins such as rhodopsins, hexose transporter, diacylglycerol kinase, ATPases, etc. The activity of these proteins (defined by the rate of photoactivation, transport, catalysis, etc.) is usually maximal for 18-carbon acyl chains, which are the predominant species in biological membranes, as a result of an optimized hydrophobic match between the protein and lipid bilayer (reviewed in ref 32). In contrast, PLA₂ and LCAT have higher activity with shorter chain PCs (7, 30), probably due to increased local disorder in a thinner bilayer (33) facilitating enzyme insertion that is necessary for lipolysis. In summary, moderate destabilization of nascent discoidal HDLs and the ensuing increase in surface disorder promote the insertion of cholesterol and LCAT, thereby facilitating efflux and esterification of cholesterol at early steps of RCT (Figure 1, steps 2 and 3).

DESTABILIZATION OF SPHERICAL HDLS PROMOTES THEIR METABOLIC REMODELING AND FUSION

Cholesterol esters produced by LCAT move from the lipoprotein surface to its core, leading to formation of mature spherical HDLs. Further remodeling of these small particles by plasma factors creates an imbalance between the apolar core and the amphipathic surface, which may lead to apolipoprotein dissociation and HDL fusion. For example, LCAT action on small spherical HDL₃ ($d = 7\text{--}9$ nm, two apoA-I molecules per particle) depletes surface cholesterol and phospholipids and produces cholesterol esters that accumulate in the core; this surface depletion and core expansion are compensated by fusion of HDL₃ into larger HDL₂ particles ($d = 10\text{--}13$ nm, three to four apoA-I

molecules) that may be accompanied by dissociation of lipid-poor apoA-I (4). Another example is CETP, which exchanges triacylglycerides from low- and very low-density lipoproteins for cholesterol esters in HDL (34). This equimolar exchange shrinks the core, since depletion of cholesterol esters exceeds enrichment with triacylglycerides that are hydrolyzed by hepatic lipase; the ensuing imbalance between the HDL core and surface induces dissociation of lipid-poor apoA-I and HDL fusion (6, 35). Furthermore, phospholipid transfer protein, which transfers phospholipids from chylomicrons and very low-density lipoproteins to HDL, also creates an imbalance between the particle core and surface, leading to HDL fusion and dissociation of lipid-poor apoA-I (36) (Figure 1, steps 4 and 5). Finally, selective uptake of HDL core lipids by SR-BI at the last step of RCT generates excess surface material, including lipid-poor apolipoproteins that re-enter RCT (5, 21) (Figure 1, step 6); this HDL disassembly resembles rupture and release of core lipids and dissociated proteins upon denaturation (19, 20). Thus, HDL fusion and rupture accompanied by apolipoprotein dissociation occur during metabolic remodeling by several plasma factors (4–6).

Since both denaturation and metabolic remodeling of mature spherical HDLs involve protein dissociation and lipoprotein fusion, these *in vitro* and *in vivo* HDL transitions may be modulated by similar kinetic barriers (19). Several lines of evidence support this notion (Table S1 of the Supporting Information). First is the role of apoA-II, a second major HDL protein with unclear function (37). The consensus is that apoA-II on spherical HDLs (i) stabilizes lipoproteins against chemical or thermal denaturation (38) and (ii) counteracts the anti-atherogenic effects of apoA-I, apparently by impeding dissociation of lipid-poor apoA-I and decelerating HDL metabolism (37, 39). This suggests that apoA-II decelerates both denaturation and metabolic remodeling of spherical HDLs, which supports our central hypothesis. Second is the effect of oxidation on HDL stability and functions. Recent studies of human plasma HDLs oxidized by copper or hypochlorite showed that mild oxidation (which involves modification of Met and certain aromatic residues on the apolar helical faces and apolipoprotein cross-linking into dimers and trimers) accelerates protein dissociation and HDL fusion and improves the ability of HDL to accept cell cholesterol via the ABCA1-mediated transport to dissociated apoA-I; this may result from the reduced affinity of mildly oxidized apolipoproteins for the lipid surface, which facilitates protein dissociation (13, 17). In contrast, advanced oxidation (which leads to massive protein cross-linking and lipolysis) prevents protein dissociation and lipoprotein fusion and weakens the ability of HDL to accept cholesterol (13, 17); this may result, in part, from lipolysis of core lipids (17).

Taken together, these studies suggest that the inverse correlation between HDL stability and the ability to undergo functional remodeling is a general trend that applies to both discoidal and spherical HDLs. Since moderate destabilization accelerates HDL remodeling at key junctures of RCT, it may benefit HDL functions in cholesterol removal. Importantly, this general trend may not always apply to specific steps in RCT that may be modulated by multiple factors. For example, the LCAT reaction requires not only disorder in HDL surface but also specific interactions between the partners (such as apoA-I, LCAT, PCs, and cholesterol). Since optimal conditions for such specificity may differ from those

which optimize surface disorder (8), structural disorder and specificity must be delicately balanced for optimal HDL functions.

FUNCTIONAL ROLE OF STRUCTURAL DISORDER IN PROTEINS AND THEIR COMPLEXES

Even though insertion of cholesterol and LCAT into HDL surface at early steps of RCT may not involve a global structural transition from intact HDL to fused particles and dissociated lipid-poor proteins, reduction of the free energy barrier for such a transition may promote local structural fluctuations in HDL surface, thereby facilitating this insertion (Figure 2B). Similarly, global protein transitions are often coupled to local structural fluctuations that modulate ligand binding (40). These and other types of intrinsic disorder have been found in an increasing number of proteins (41–44), including those involved in cardiovascular disease (24, 26, 44). Intrinsic disorder in these proteins is thought to be functionally important for binding to the phospholipid surface, high-specificity/low-affinity binding, specific binding to multiple targets, rapid protein turnover, allosteric regulation, and signal transduction (24, 40–44). We propose that intrinsic disorder is also important for functions of macromolecular assemblies that are stabilized by kinetic barriers, such as lipoproteins.

In summary, moderate structural disorder accelerates metabolic remodeling of HDLs, which may be beneficial for HDL functions in cholesterol removal. Therefore, HDL stability must be delicately balanced to maintain the structural integrity of the lipoprotein assembly and ensure structural specificity necessary for interactions of HDL with its metabolic partners yet enable efficient efflux and processing of cell cholesterol and rapid HDL remodeling and turnover at key junctures of RCT.

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SUPPORTING INFORMATION AVAILABLE

References that support the correlation between structural stability and functions of HDL (Table S1) and thermal denaturation data of apoA-I complexes with PCs (Figures S1–S3). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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