Interactions of Apolipoprotein A-I with High-Density Lipoprotein Particles

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ABSTRACT: Although the partitioning of apolipoprotein A-I (apoA-I) molecules in plasma between high-density lipoprotein (HDL)-bound and -unbound states is an integral part of HDL metabolism, the factors that control binding of apoA-I to HDL particles are poorly understood. To address this gap in knowledge, we investigated how the properties of the apoA-I tertiary structure domains and surface characteristics of spherical HDL particles influence apoA-I binding. The abilities of 14C-labeled human and mouse apoA-I variants to associate with human HDL and lipid emulsion particles were determined using ultracentrifugation to separate free and bound protein. The binding of human apoA-I (243 amino acids) to HDL is largely mediated by its relatively hydrophobic C-terminal domain; the isolated N-terminal helix bundle domain (residues 1–190) binds poorly. Mouse apoA-I, which has a relatively polar C-terminal domain, binds to human HDL to approximately half the level of human apoA-I. The HDL binding abilities of apoA-I variants correlate strongly with their abilities to associate with phospholipid (PL)-stabilized emulsion particles, consistent with apoA-I–PL interactions at the particle surface being important. When equal amounts of HDL2 and HDL3 are present, all of the apoA-I variants partition preferentially to HDL3. Fluorescence polarization measurements using Laurdan-labeled HDL2 and HDL3 indicate that PL molecular packing is looser on the more negatively charged HDL3 particle surface, which promotes apoA-I binding. Overall, it is clear that both apoA-I structural features, especially the hydrophobicity of the C-terminal domain, and HDL surface characteristics such as the availability of free space influence the ability of apoA-I to associate with HDL particles.

High-density lipoprotein (HDL) possesses anti-atherogenic properties that arise, in part, from its participation in the reverse cholesterol transport pathway in which the principal protein, apolipoprotein A-I (apoA-I), plays a central role.1,2 As a result, there is great interest in understanding the structure–function relationships of apoA-I (for reviews, see refs 3–6). ApoA-I in a double-belt arrangement stabilizes discoidal HDL particles7,8 and in a trefoil arrangement, which has similar protein–protein contacts, stabilizes spherical HDL particles.9 The apoA-I molecules in HDL particles are in a highly dynamic state,10,11 and besides participating in the “scaffold” structures that control particle stability described above, apoA-I also exists in a labile pool that can dissociate from the particle surface and exchange between different HDL particles.12–15 This exchangeable pool is a precursor of lipid-free (poor) apoA-I or pre-β1 HDL16 that plays a key role in promoting ABCA1-mediated efflux of cellular phospholipid (PL) and cholesterol, and formation of nascent HDL particles.17 Higher levels of circulating pre-β1 HDL are apparently cardioprotective,18 so knowledge of how to increase the size of the precursor pool of apoA-I molecules on spherical HDL particles could be beneficial. It follows that understanding the factors that control binding of apoA-I to HDL particles is significant. Previously, we used surface plasmon resonance (SPR) to show that the binding of apoA-I to HDL is reversible and that protein–protein as well as protein–phospholipid interactions are involved.19

Here we explore in detail the influences of apoA-I tertiary structure domain properties and spherical HDL surface characteristics on the binding process. Also, by comparing the binding of apoA-I variants to a lipid emulsion on one hand and HDL on the other, we address the question of how the lipid binding capabilities of apoA-I influence binding to the lipoprotein particle. The results demonstrate that the hydrophobicity of the C-terminal domain of human apoA-I and the availability of free space in the surface of HDL particles play critical roles in determining the degree of apoA-I binding. These findings are pertinent to how other minor protein
components, detected by proteomic analysis, associate with HDL particles and are transported in plasma.

**EXPERIMENTAL PROCEDURES**

**Materials.** HDL$_2$ (1.065 g/mL < d < 1.125 g/mL) and HDL$_3$ (1.125 g/mL < d < 1.21 g/mL) were isolated by sequential ultracentrifugation from a pool of normolipidemic frozen human plasma obtained by combining several single units. These preparations were characterized as described previously. HDL was delipidated in an ethanol/diethyl ether mixture, and purified apoA-I$^{22}$ and apoA-II$^{23}$ were isolated by anion exchange chromatography on Q-Sepharose. Human and mouse apoA-I and engineered variants were expressed as thiorexin fusion proteins in *Escherichia coli* and isolated as reported previously.$^{28,29}$ Cleavage of the thiorexin fusion protein with thrombin leaves the target apoA-I with two extra amino acids, Gly and Ser, at the amino terminus. The preparation and characterization of the full-length proteins, their isolated N- and C-terminal domains, and human–mouse hybrid apoA-I molecules have been described previously.$^{28,30,31}$ The human apoA-I variants containing C-terminal mutations at positions 225, 229, 232, and 236 have also been described previously.$^{32}$ The apoA-I preparations were at least 95% pure as assessed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). The proteins were stored as lyophilized powders at $-20^\circ$C and, prior to being used, were dissolved in 6 M GdnHCl and dialyzed extensively at $4^\circ$C against the appropriate buffer. Protein concentrations were determined by either a modified Lowry procedure$^{33}$ or the enzymatic assay kits from Thermo Scientific (Middleton, VA) and Wako (Richmond, VA), respectively. The triolein:PC molar ratio in the emulsion after isolation by ultracentrifugation was 4.6 ± 0.3:1, and the average particle diameter determined by quasi-elastic light scattering was 86 ± 7 nm. The binding of apoA-I was monitored by incubation of the $^{14}$C-labeled protein with the emulsion for 1 h at room temperature and separation of free and bound apoA-I by centrifugation, as described previously.$^{38}$

**HDL Modification.** The apoA-II contents of HDL$_2$ and HDL$_3$ were increased by incubation with a 1.2:1 (w/w) apoA-II:HDL protein ratio in TBS for 2 h at room temperature.$^{39-41}$ After reisolation of the HDL by ultracentrifugation, the protein:phospholipid ratio (w/w) was measured. As a result of apoA-II binding with displacement of most of the apoA-I (monitored by SDS–PAGE), the protein:phospholipid ratios of the HDL$_2$ and HDL$_3$ preparations increased by $\sim$45%. LpA-I and LpA-I with A-II were isolated from HDL by covalent chromatography on thiopropyl Sepharose.$^{42,43}$ The Lp-A-I samples obtained in this fashion contained <5% apoA-II as assessed via SDS–PAGE. The HDL surface charge was modified by acetylation of protein lysine residues with acetic anhydride.$^{44,45}$ In brief, HDL in a sodium acetate solution on ice was incubated while being stirred for several hours with 1.5 times its mass on a protein basis of acetic anhydride. After dialysis, the acetylated HDL$_2$ and HDL$_3$ preparations were subjected to electrophoresis in agarose gels to determine the surface potentials of the particles. The negative surface potential of unmodified HDL$_3$ was $\sim$15% higher than that of unmodified HDL$_2$, and for both HDL fractions, acetylation increased the negative surface charge by 30–40% (data not shown).

**Fluorescence Spectroscopy.** HDL samples were labeled with Laurdan via addition of small aliquots of a stock solution of Laurdan in dimethyl sulfoxide to yield a PL:probe molar ratio of 100:1.$^{47,48}$ After incubation for 1 h at 37 $^\circ$C to equilibrate the probe with HDL, steady-state Laurdan fluorescence emission spectra were collected with a Hitachi F-4500 fluorescence spectrophotometer. The generalized polarization (GP) value was calculated from the emission intensities using the equation $GP = (I_B - I_F/I_B + I_F)$, where $I_B$ and $I_F$ are the emission intensities at the blue (425 nm) and red (485 nm) edges of the emission spectrum, respectively.$^{49}$

**RESULTS**

In an earlier study, we used SPR to monitor the kinetics of reversible interaction of apoA-I with HDL particles and determine the mechanism of binding.$^{50}$ The results are consistent with apoA-I interacting with both PL and protein sites on the HDL particle surface. To improve our understanding of the contributions of these two types of interactions and the reasons for differential binding to the HDL$_2$ and HDL$_3$ subclasses, we now compare the binding of apoA-I variants with altered tertiary structural domain characteristics to HDL and lipid emulsion particles.

**Influence of ApoA-I Structure on HDL Binding.** Figure 1 compares the binding of human and mouse apoA-I and their N- and C-terminal tertiary structure domains when added to a mixture containing equal amounts (on a protein basis) of HDL$_2$ and HDL$_3$. In agreement with prior SPR data,$^{17}$ the N-terminal helix bundle domain (residues 1–189) of human apoA-I binds relatively poorly compared to the intact protein. In marked contrast, the relatively hydrophobic C-terminal domain
(residues 190–243) binds like the intact protein; this domain bound poorly in the SPR experiments presumably because at the higher concentrations used it was self-associated. The more hydrophobic human apoA-II molecule binds to HDL somewhat better than apoA-I (white) with the K225-A226 sequence characteristic of FVB mice agrees with a recent report. The data in Figure 1B indicate that the N- and C-terminal domains of mouse apoA-I possess similar HDL binding capabilities, unlike the situation for human apoA-I in which the C-terminal domain binds much better.

Figure 2 compares the abilities of the proteins described in the legend of Figure 1 to bind to lipid emulsion particles. Comparison of Figures 1A and 2A shows that the abilities of the human apoA-I domains and apoA-II to bind to a lipid emulsion parallel their abilities to bind to HDL. The observation that, relative to the intact human apoA-I molecule, the isolated N-terminal domain (residues 1–189) binds poorly to lipid emulsion particles agrees with a prior report from this laboratory. The fractional binding values are plotted as means ± SD (n ≥ 3). Application of the statistical test described for Figure 1 indicated that the fractional binding of all proteins was significantly different (p < 0.01) from the reference value for human apoA-I.

Figure 2. Binding of human and mouse apoA-I, the N- and C-terminal domains of apoA-I, and human apoA-II to lipid emulsion particles. The binding assay described in Experimental Procedures was used to determine the fraction of each apoA-I variant that bound to the egg PC/triolein emulsion particles, and this value is normalized to the fraction (50 ± 2%) of human apoA-I bound. The designations of the proteins in panels A and B are the same as in the legend of Figure 1. The fractional binding values are plotted as means ± SD (n ≥ 3). Application of the statistical test described for Figure 1 indicated that the fractional binding of all proteins was significantly different (p < 0.01) from the reference value for human apoA-I.
this case, the isolated N-terminal domain (residues 1–186) binds somewhat better than the intact protein. The isolated C-terminal domain (residues 187–240) binds poorly, explaining why its presence in the intact mouse apoA-I molecule gives rise to relatively limited lipid emulsion binding. It is noteworthy that, while the emulsion binding ability of mouse apoA-I residues 187–240 is poor (Figure 2B), the HDL binding ability of this segment is more similar to that of the intact mouse apoA-I molecule (Figure 1B). However, the HDL and lipid emulsion binding abilities of all the proteins described in the legends of Figures 1 and 2 are correlated (Figure 3); this effect is expected because interaction of apoA-I with the PL-covered surface occurs with both types of particles. The \( r^2 \) value indicates that \( \sim70\% \) of the variance between the apoA-I molecules in HDL binding is due to differences in PL binding ability. The lack of a perfect correlation between HDL and emulsion binding abilities presumably reflects the contribution of apoA-I–resident protein interactions to HDL binding.

To further explore how apoA-I tertiary structure domain properties influence the ability to bind to HDL particles, we compared the HDL and emulsion binding behaviors (Figure 4) of some previously characterized human–mouse apoA-I hybrids in which the N- and C-terminal domains are swapped.\(^{30,31}\) The HDL and emulsion binding results for the apoA-I molecules described in panels A and B of Figure 4 are highly correlated (\( r^2 = 0.89 \) (data not shown)). The results in Figure 4A demonstrate that substitution of either the mouse C-terminal domain (residues 187–240) or the C-terminal segment (residues 218–240) in the human apoA-I molecule reduces the level of HDL binding by approximately 50%, whereas substitution of the human apoA-I C-terminal domain (residues 190–243) or segment (residues 221–243) into mouse apoA-I enhances its HDL binding ability. These substitutions have similar effects on emulsion binding (Figure 4B). Introduction of the mouse C-terminal residues into human apoA-I causes a larger reduction in the level of emulsion binding (\( \sim70\% \)) of the human–mouse apoA-I hybrids, consistent with the poor lipid binding ability of this region of the mouse apoA-I molecule (Figure 2B).\(^{30,31}\) Overall, the results in Figure 4 demonstrate the important role played by the C-terminal domain and \( \alpha \)-helix in apoA-I HDL and lipid emulsion binding. It is evident that the presence of the more hydrophobic human apoA-I C-terminal amino acids enhances binding to both types of particles.

Given the importance of the human apoA-I C-terminal domain that is the most hydrophobic region of the protein,\(^{54,55}\) experiments summarized in Figure 5 were conducted to examine in more detail how alterations in hydrophobicity influence HDL and emulsion binding. As seen for the proteins studied in Figures 1 and 4, the HDL and lipid emulsion binding behaviors of the apoA-I variants described in Figure 5 are linearly correlated (\( r^2 = 0.72 \) (data not shown)). Consistent with the entire C-terminal domain being required for effective lipid binding,\(^{55,56}\) deletion of residues 190–220 (variant Δ190–220) reduces the level of binding to HDL and lipid emulsion by 50–60%, and removal of residues 223–243 (variant 1–222) has similar effects (cf. panels A and B of Figure 5). Substitution of the aromatic residues in the C-terminal helix with leucine and alanine residues (variant F225L/F229A/...
Y236A) reduces the hydrophobicity and reduces the level of both HDL and emulsion binding, with the reduction in the level of emulsion binding being larger. Restoration of the C-terminal helix hydrophobicity to the level for human apoA-I by substitution of leucine residues (variant F225L/F229L/A232L/Y236L) also restores the HDL and emulsion binding abilities (Figure 5A,B). These manipulations of the hydrophobicity of the human apoA-I C-terminal helix induce parallel effects on the ability of the protein to solubilize dimyristoyl PC multilamellar vesicles.

**Influence of HDL Structure on ApoA-I Binding.** The ultracentrifugation assay described in Experimental Procedures gives information about the relative binding of apoA-I to HDL

As shown in Figure 6A, when a small amount of apoA-I is added to a mixture containing equal amounts of HDL	extsubscript{2} and HDL	extsubscript{3} (on a protein basis), human apoA-I and its N- and C-terminal domains bind more to HDL	extsubscript{3}. In the case of the intact human apoA-I molecule, the level of binding to HDL	extsubscript{3} is approximately twice that to HDL	extsubscript{2}. Removal of the C-terminal domain (variant 1–189) causes a larger reduction in the level of binding to HDL	extsubscript{3} than to HDL	extsubscript{2}, so that the relative partitioning to HDL	extsubscript{3} is enhanced. A relative enhancement in binding to HDL	extsubscript{3} is also observed with the isolated C-terminal domain (variant 190–243) (Figure 6A). The data in Figure 6B show that either removal of the C-terminal segment (variant 1–222) or a decrease in the hydrophobicity of this segment (variant F225L/F229A/Y236A) reduces the level of binding of human apoA-I.
apoA-I to HDL₂ more than to HDL₃. Restoration of the C-terminal segment hydrophobicity (variant F225L/F229L/A232L/Y236L) restores the relative HDL₂–HDL₃ binding to that seen with wild-type human apoA-I. Substitution of either the human C-terminal domain with the relatively polar mouse apoA-I counterpart (variant Hu1–189/Mo187–240) or the mouse apoA-I C-terminal sequence in human apoA-I (variant Hu1–220/Mo218–240) reduces the level of binding to HDL₂ and HDL₃ similarly and does not have much effect on the relative binding to the two HDL subclasses (Figure 6C). Mouse apoA-I gives rise to differently sized HDL particles compared to those with human apoA-I, and the two species of apoA-I can be distributed differently between HDL₂ and HDL₃, with the segment of the protein spanning residues 165–209 contributing to this effect.35,57 We compared the HDL₂ versus HDL₃ distributions of the isolated mouse apoA-I N- and C-terminal domains together with those of mouse–human apoA-I hybrids (Figure 7) to determine the influence, if any, of these structural domains on HDL₂ versus HDL₃ partitioning. Under the conditions of our assay that contains equal amounts of HDL₂ and HDL₃, mouse apoA-I binds relatively well to HDL₃. This result is in contrast to an earlier finding that mouse apoA-I binds more to HDL₃ than HDL₂.55 The discrepancy is presumably a consequence of different experimental conditions. The isolated N- and C-terminal domains of mouse apoA-I partition more to HDL₃ than to HDL₂, with the relative preference for HDL₃ being weaker for the N-terminal domain (variant 1–186). As expected, introduction of either the hydrophobic human apoA-I C-terminal domain or segment to give the Mo1–186/Hu190–243 and Mo1–217/Hu221–243 apoA-I hybrids enhances binding (relative to the binding of mouse apoA-I) to both HDL₂ and HDL₃. The enhancement of apoA-I binding is greater with HDL₂, so that these hybrids partition approximately equally between HDL₂ and HDL₃ (Figure 7). Overall, the data in Figure 7 indicate that the properties of the C-terminal domain or segment of apoA-I influence the relative binding to HDL₂ and HDL₃.

Our prior SPR experiments demonstrated that binding of apoA-I to HDL involves, in part, protein–protein interactions, and because HDL₃ has a higher ratio of apoA-II to apoA-I than HDL₂,58,59 we tested the hypothesis that increased apoA-I–apoA-II interactions are responsible for the enhanced binding of apoA-I to HDL₃ (Figure 6). We separated the LpA-I and LpA-I with A-II fractions from HDL₂ and HDL₃ and compared the binding of apoA-I to each. The presence of apoA-II did not affect the partitioning of apoA-I between the HDL₂ and HDL₃ fractions (data not shown). To further explore any potential effects of the presence of apoA-II on the HDL particle surface, we pretreated HDL with apoA-II to displace apoA-I and create apoA-II-enriched HDL particles. The protein content relative to the content of PL in these particles treated in this fashion is increased (cf. Experimental Procedures), and rather than enhanced apoA-I binding, the binding to HDL₃ is not significantly affected and the level of binding to A-II-HDL₂ greatly reduced (Figure 8). It follows that the higher apoA-II content of HDL₃ relative to that of HDL₂ is not the reason for apoA-I binding better to HDL₃. Furthermore, the enrichment of the HDL₂ particle surface with apoA-II reduces the level of apoA-I binding, presumably by eliminating free space in the surface.

Another property of the HDL particle that may affect apoA-I binding is the net surface charge. The surface potentials of HDL₂ and HDL₃ are different, with the latter particle being more negatively charged.56 To test the concept that the extra negative charge on HDL₃ promotes the binding of apoA-I molecules, we increased the negative charge at the HDL particle surface by acetylating the lysine residues on resident proteins (cf. Experimental Procedures) and examined the consequences for apoA-I binding. The greater negative surface charge of acetylated HDL₂ and HDL₃ increases the level of binding of apoA-I to both particles (Figure 9A). A possible explanation for this effect is that the increased net negative charge disorders the HDL particle surface, thereby creating additional free space into which apoA-I molecules can adsorb. To test this concept, we measured the Laurdan generalized polarization (GP) in HDL particles containing this fluorescent probe on the surface (cf. Experimental Procedures). A lower value of GP indicates less PL order (looser molecular packing)

Figure 7. Relative abilities of mouse apoA-I variants to bind to HDL₂ and HDL₃. The experimental data were obtained and are presented as described in the legend of Figure 6. The white and hatched bars refer to binding to HDL₂ and HDL₃, respectively. The mouse apoA-I (white) and the N- and C-terminal domains have been identified in the legend of Figure 1B. The mouse–human hybrid apoA-I molecules are identified in the legend of Figure 4. The fractional binding values are plotted as means ± SD (n ≥ 6). ANOVA followed by a Dunnett’s multiple-comparison test indicated that the fractional binding of all proteins was significantly different (p < 0.01) from the reference value for human apoA-I binding to HDL₃.
in the PL monolayer. The Laurdan GP is lower in HDLț than in HDLŴ (Figure 9B), indicating that the PL order is lower in the former particle. The greater free space in the HDLț particle surface leads to enhanced apoA-I binding. Consistent with this idea, acetylation of HDLŴ and HDLț reduces the Laurdan GP, with the effect being larger for HDLŴ (Figure 9B), reflecting a reduction in PL packing density that leads to enhanced apoA-I binding (Figure 9A).

**DISCUSSION**

**Influence of ApoA-I Structure on HDL Binding.** Deletion of the C-terminal domain weakens the ability of human apoA-I to bind to HDL, indicating that this domain plays a critical role in mediating the initial interaction with the HDL surface. Such processes is followed by a second step in which the N-terminal helix bundle domain opens to allow helix–PL interactions (reviewed in refs 6 and 55). The strong HDL binding properties of the C-terminal domain are demonstrated by the fact that this domain in isolation binds as well as the intact apoA-I molecule (Figure 1A). This ability of the human apoA-I C-terminal domain to bind well to HDL is a consequence of its hydrophobicity, and increasing the hydrophobicity of this domain increases the level of HDL binding (Figure 5A). Conversely, apoA-I molecules possessing a relatively polar C-terminal domain, such as the one that occurs in mouse apoA-I and the hybrid human–mouse apoA-I molecules described in Figure 4A, bind less well to HDL. These results and the observation that the relatively nonpolar human apoA-II molecule binds well (Figure 1A) together demonstrate clearly that hydrophobic interactions promote binding of apoA-I to HDL. These interactions occur as the nonpolar faces of the apoA-I amphipathic α-helices insert among the PL molecules present as a monomolecular film at the HDL particle surface. This apoA-I–PL interaction occurs on the surfaces of both PL-stabilized emulsion particles and HDL particles, explaining the strong correlation in the abilities of apoA-I variants to bind to both types of particles (Figure 3). However, binding of apoA-I to HDL particles can also involve protein–protein interactions. Such interactions are responsible for the isolated mouse C-terminal domain, which is relatively polar and has very limited ability to bind to a PL-stabilized emulsion (Figure 2B), binding to HDL essentially as the intact mouse apoA-I molecule (Figure 1B).

**Influence of HDL Structure on ApoA-I Binding.** The results in Figures 6 and 7 indicate that most of the apoA-I variants bind more to HDLț than to HDLŴ when both lipoproteins are present at the same protein concentration. Under this condition, it can be estimated from the dimensions and compositions of the spherical HDLŴ and HDLț particles that the total surface area of each type of particle in the mixture is similar (the total HDLŴ surface area is ~10% greater). It is of interest to understand the mechanisms responsible for the preferential binding of apoA-I to HDLț. Estimates of the areas occupied by the PL and protein constituents present at the
particle surface indicate that ~30 and ~20%, respectively, of the surfaces of HDL₃ and HDL₄ particles are PL-covered. The greater availability of PL-covered surface in HDL₂ that can give rise to enhanced nonpolar apoA-I helix−PL interactions is presumably the reason that, relative to the HDL₁ case, binding to HDL₂ is more affected by changes in apoA-I C-terminal segment hydrophobicity (Figure 6B). The greater enhancement of binding of mouse−human apoA-I hybrids containing either the human C-terminal domain or segment to HDL₂ compared to HDL₁ (Figure 7) is most likely also due to hydrophobic apoA-I−PL interactions being more important for HDL₂ binding than for HDL₁ binding. Obviously, because the HDL₂ and HDL₁ particle surfaces are mostly occupied by protein, polar and nonpolar protein−protein interactions can also occur readily when an apoA-I molecule binds. Because apoA-I−apoA-II attractive interactions have been demonstrated, it is possible that the higher apoA-II content of HDL₁ might be responsible for the observed binding preference of apoA-I for this particle. However, this seems not to be the case because apoA-I binds equally well to LpA-I and LpA-I with A-II HDL particles, and enrichment of HDL₂ and HDL₁ particles with apoA-II does not increase the level of binding. Indeed, this enrichment with apoA-II inhibits apoA-I binding (Figure 8). The simplest explanation of this effect is that the higher protein content on the HDL particle surface reduces the amount of free space available to apoA-I.

If greater availability of free space at the particle surface is the reason for apoA-I binding more to HDL₃ than to HDL₂, it is to be expected that PL molecular packing is looser on the HDL₁ particle surface. The Laurdan fluorescence polarization results (Figure 9) demonstrate the PL order is indeed lower in HDL₃ than in HDL₂, explaining why apoA-I binds relatively well to HDL₃. This finding is consistent with comparisons between different classes of lipoproteins showing that increased surface fluidity enhances the reactivity to lipolytic enzymes. Why is the HDL₃ particle surface more disordered than that of HDL₂? The net negative surface charge is higher for HDL₁ particles and the resultant strengthened electrostatic repulsion may lower the molecular packing density. Altering HDL particle surface charge does affect the molecular structure because acetylation increases the net negative charge, thereby decreasing the PL order, especially in the case of HDL₂. This change in PL molecular packing allows an increased level of apoA-I binding (Figure 9). Thus, HDL₁ is a better substrate than HDL₃ for apoA-I binding because the greater net negative charge gives rise to a lower molecular packing density and more free interfacial space into which apoA-I molecules can adsorb.

Most of the apoA-I on the surface of spherical HDL particles is organized as a stabilizing scaffold but a labile pool of easily dissociable apoA-I molecules also exists. The current observation that the surface structure of HDL₃ favors apoA-I binding suggests that there should be a larger pool of weakly bound apoA-I on this HDL subclass, as has been observed. This observation coupled with the fact that in normal human plasma the concentration of apoA-I in HDL₃ is greater than that in HDL₂ suggests that HDL₃ is the major source of dissociable apoA-I; this apoA-I is the precursor for pre-β₁-HDL that plays a key role in reverse cholesterol transport. Because apoA-I interacts through protein−PL and protein−protein interactions at the HDL particle surface, it is likely that the many minor protein constituents of HDL detected by proteomics analysis also interact similarly and become transported in plasma as part of HDL. There is evidence that, compared to HDL₂, the HDL₃ subclass contains relatively more of these minor proteins.

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Notes
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ABBREVIATIONS

ABCA1, ATP binding cassette transporter A₁; apo, apolipoprotein; GdnHCl, guanidinium hydrochloride; GP, generalized polarization; HDL, high-density lipoprotein; PC, phosphatidylcholine; PL, phospholipid; SPR, surface plasmon resonance.

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