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

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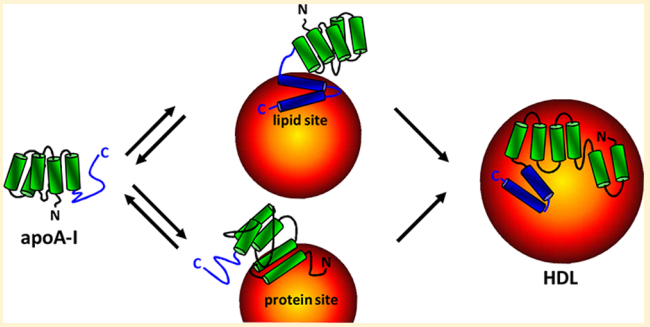
ABSTRACT: Although the partitioning of apolipoprotein A-I (apoA-I) molecules in plasma between high-density lipoprotein (HDL)-bound and -unbound states is an integral part of HDL metabolism, the factors that control binding of apoA-I to HDL particles are poorly understood. To address this gap in knowledge, 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 ^{14}C-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 HDL_2 and HDL_3 are present, all of the apoA-I variants partition preferentially to HDL_3. Fluorescence polarization measurements using Laurdan-labeled HDL_2 and HDL_3 indicate that PL molecular packing is looser on the more negatively charged HDL_3 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 particles[7,8] 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,[6,10] 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.[11−15] This exchangeable pool is a precursor of lipid-free (poor) apoA-I or pre-β1 HDL[16] 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 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

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components, detected by proteomic analysis,[20,21] 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[22] from a pool of normolipidemic frozen human plasma obtained by combining several single units. These preparations were characterized as described previously.[23] HDL was delipidated in an ethanol/diethyl ether mixture,[24] and purified apoA-I[25] and apoA-II[26] were isolated by anion exchange chromatography[27] on Q-Sepharose. Human and mouse apoA-I and engineered variants were expressed as thioredoxin fusion proteins in *Escherichia coli* and isolated as reported previously.[28,29] Cleavage of the thioredoxin fusion protein with thrombin leaves the target apoA-I with two extra amino acids, Gly and Ser, at the amino terminus. The preparation and chacterization 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 °C and, prior to being used, were dissolved in 6 M GdnHCl and dialyzed extensively at 4 °C against the appropriate buffer. Protein concentrations were determined by either a modified Lowry procedure[33] or the absorbance at 280 nm. Egg yolk phosphatidylcholine (PC) and triolein were purchased from Avanti Polar Lipids (Birmingham, AL) and Sigma (St. Louis, MO), respectively. Laurdan was obtained from Molecular Probes (Eugene, OR).

Binding of ApoA-I to HDL_2 and HDL_3. The apoA-I preparations were labeled with ^{14}C to specific activities of ∼2 μCi/mg of protein by reductive methylation of lysine residues using [^{14}C]formaldehyde, as described previously;[34] this level of trace labeling does not alter the lipid affinity of apolipoproteins. A mixture containing 0.45 mg of HDL_2 and 0.45 mg of HDL_3 (on a protein basis) and 5 μg of [^{14}C]apoA-I in 1.0 mL of TBS (pH 7.4) at 4 °C was prepared. Under this condition, there is insignificant remodeling of the HDL particles because of the binding of apoA-I.[35] Three 10 μL aliquots were removed, and the total initial ^{14}C radioactivity was determined by liquid scintillation counting using Scintiverse. The mixture was incubated for 30 min at 4 °C and then mixed with 7 mL of a cold KBr solution to adjust the density to 1.125 g/mL. The HDL_2 (top 1.5 mL) was isolated by ultracentrifugation at 4 °C (24 h at 50000 rpm in a Beckman 70.1 Ti rotor). The density of the remaining solution was adjusted to 1.21 g/mL by addition of solid KBr, and the HDL_3 (top 2.0 mL) was separated from the bottom fraction (unbound apoA-I) by respinning. The isolated fractions were dialyzed against TBS to remove KBr using Slide-A-Lyzer mini dialysis units (3500 molecular weight cutoff) (Pierce) before liquid scintillation counting was used to determine the distribution of [^{14}C]apoA-I between HDL_2, HDL_3, and the unbound fractions. The recovery of HDL in each fraction was determined by measuring the protein concentration.

Binding of ApoA-I to Lipid Emulsion Particles. Emulsion particles were prepared by sonication of a triolein/egg yolk PC mixture (3.5:1, w/w) in TBS (pH 7.4).[36,37] The triolein and PC contents of the emulsion were measured using 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 ∼45%. LpA-I and LpA-I with A-II were isolated from HDL by covalent chromatography on thiopropyl Sepharose.[42,43] The LpA-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.[46] The negative surface potential of unmodified HDL_3 was ∼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 °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 $GP = (I_B - I_R/I_B + I_R)$, where I_B and I_R 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.[19] 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,[19] 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

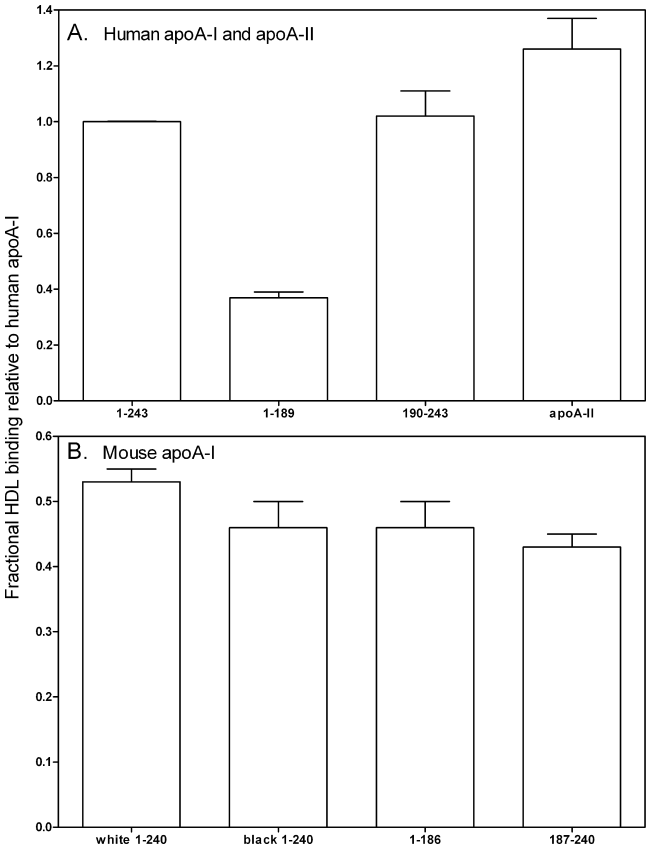

Figure 1. Binding of human and mouse apoA-I, the N- and C-terminal domains of apoA-I, and human apoA-II to HDL. The binding assay described in Experimental Procedures was used to determine the fraction of each apoA-I variant that bound to the HDL_2 and HDL_3 mixture, and this value is normalized to the fraction (68 ± 2%) of human apoA-I bound. (A) Relative binding of human apoA-I (residues 1−243), the N-terminal helix bundle domain (residues 1−189), the C-terminal domain (residues 190−243), and apoA-II. (B) Relative binding of mouse apoA-I (residues 1−240) with the K225-A226 sequence characteristic of FVB mice (white) and the Q225-V226 sequence characteristic of C57BL/6 mice (black). The relative binding of the N-terminal domain (residues 1−186) and the C-terminal domain (residues 187−240 with the K225-A226 sequence) is also shown. All values are plotted as means ± the standard deviation (SD); the numbers of measurements (n) are 63 and 30, respectively, for human apoA-I (residues 1−243) and mouse apoA-I (residues 1−240). For the apoA-I variants, the number of values is at least six. One-way analysis of variance (ANOVA) followed by a Dunnett's multiple-comparison test using Graphpad Prism 4.0 indicated that, apart from the human apoA-I C-terminal domain of residues 190−243, the fractional binding of all the other proteins was significantly different ($p < 0.01$) from the reference value for human apoA-I. Direct comparison of the values for apoA-I from the two mouse strains by an unpaired t test indicated that white apoA-I binds significantly more ($p < 0.0001$) than black apoA-I.

(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.[50] The more hydrophobic human apoA-II molecule[39,40,51,52] binds to HDL somewhat better than apoA-I (Figure 1A). The N- and C-terminal domains of mouse apoA-I (residues 1−186 and 187−240, respectively) have characteristics different from those of their human apoA-I counterparts,[30] leading to different HDL binding abilities. As shown in Figure 1B, mouse apoA-I and its isolated N- and C-terminal domains bind approximately half as well as human apoA-I. The observation of a reduced level of binding of mouse apoA-I to human HDL is consistent with prior reports.[19,35] Furthermore, the finding that apoA-I (black) containing the C-terminal Q225-V226 amino acid sequence characteristic of C57BL/6 mice binds to human HDL less well than apoA-I (white) with the K225-A226 sequence characteristic of FVB mice agrees with a recent report.[53] 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.

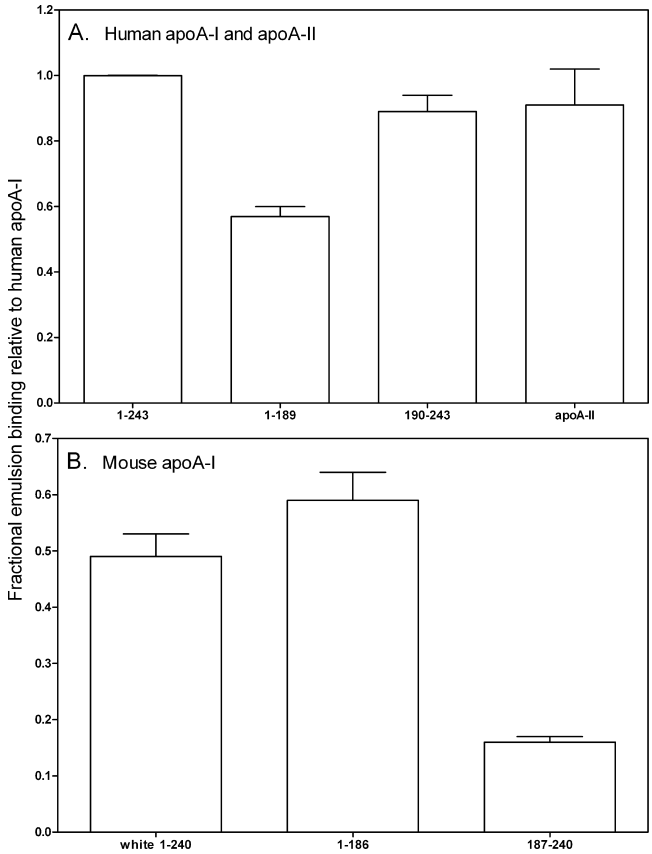

Figure 2. Binding of human and mouse apoA-I, the N- and C-terminal domains of apoA-I, and human apoA-II to lipid emulsion particles. The binding assay described in Experimental Procedures was used to determine the fraction of each apoA-I variant that bound to the egg PC/triolein emulsion particles, and 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 ($n \geq 3$). 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.

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.[38] Mouse apoA-I binds approximately half as well as human apoA-I to lipid emulsion particles (Figure 2B), but in

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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

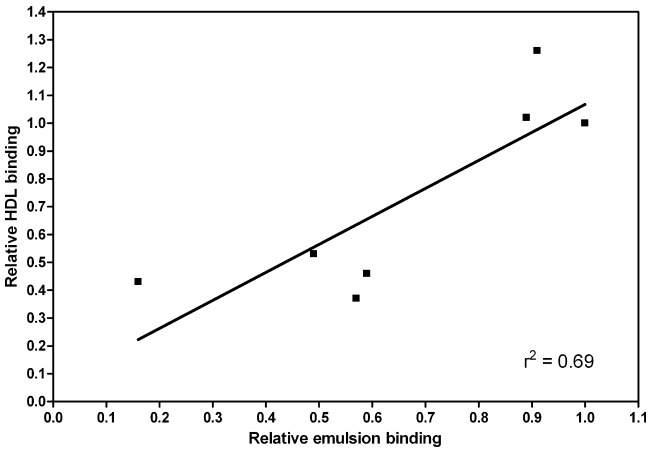

Figure 3. Correlation of the HDL and emulsion binding capabilities of human and mouse apoA-I variants, and apoA-II. The relative binding data from Figures 1 and 2 are plotted and fit by linear regression.

is expected because interaction of apoA-I with the PL-covered surface occurs with both types of particles. The r^2 value indicates that ∼70% 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 (∼70%) 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 α-helix in apoA-I HDL and lipid emulsion binding. It is evident that the presence of the more

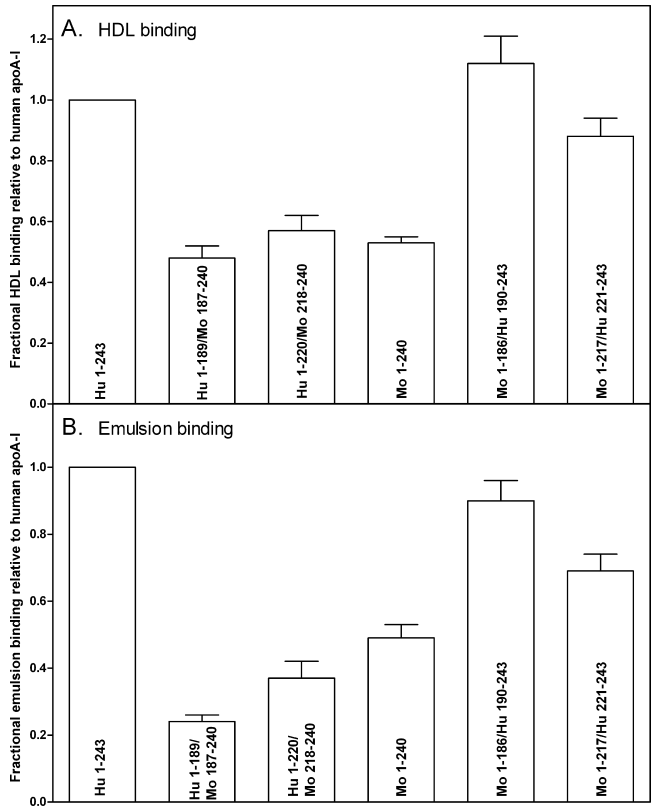

Figure 4. Influence of the apoA-I C-terminal domain and α-helix on HDL and lipid emulsion binding ability. The relative binding abilities of the apoA-I variants were determined as described in the legends of Figures 1 and 2. (A) Relative HDL binding of human apoA-I (Hu 1−243), the human−mouse apoA-I hybrid containing the human N-terminal domain and the mouse C-terminal domain (Hu 1−189/Mo 187−240), the human−mouse apoA-I hybrid with the human C-terminal segment swapped for the mouse (white) sequence (Hu 1−220/Mo 218−240), mouse (white) apoA-I, the mouse−human apoA-I hybrid containing the mouse N-terminal domain and the human C-terminal domain, and a mouse−human apoA-I hybrid with the mouse C-terminal segment swapped for the human sequence (Mo 1−217/Hu 221−243). (B) Relative emulsion binding of the same proteins as listed for panel A. The fractional binding values are plotted as means ± SD ($n \geq 6$). For the data in both panels, 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.

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,[6,55,56] 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/

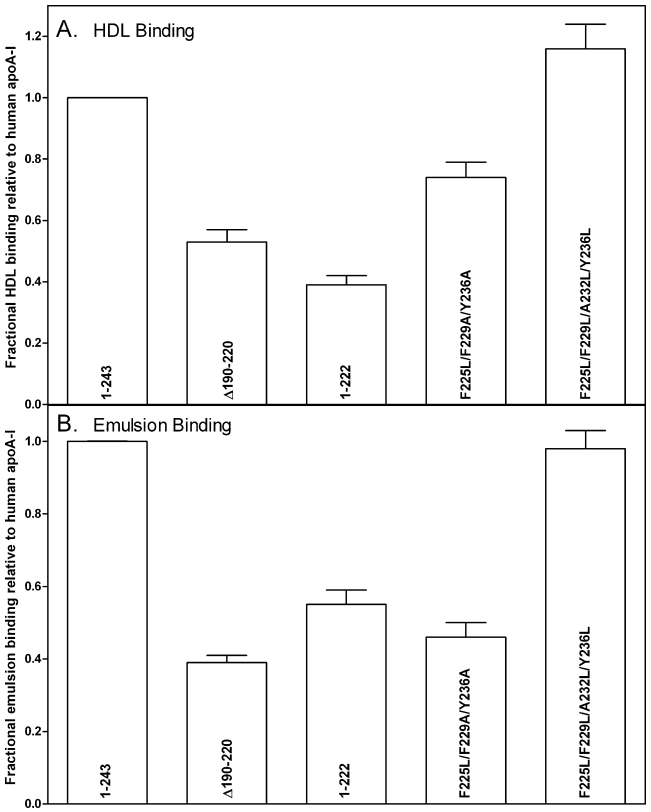

Figure 5. Influence of apoA-I C-terminal domain shortening and altered hydrophobicity on HDL and lipid emulsion binding ability. The relative binding abilities of the apoA-I variants were determined as described in the legends of Figures 1 and 2. (A) Relative HDL binding of human apoA-I (1−243), apoA-I (Δ190−220), apoA-I (1−222), apoA-I (F225L/F229A/Y236A), and apoA-I (F225L/F229L/A232L/Y236L). (B) Relative emulsion binding of the same proteins .listed for panel A. The fractional binding values are plotted as means ± SD ($n \geq 3$). For the data in both panels, 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.

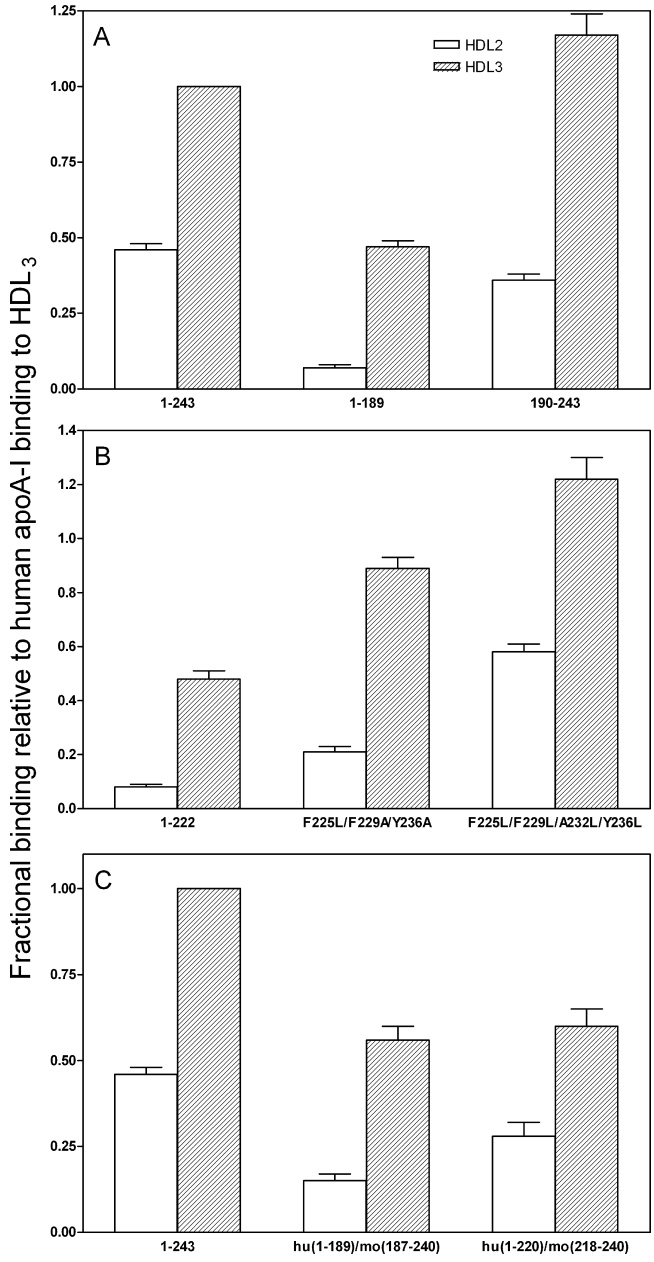

Figure 6. Relative abilities of human apoA-I variants to bind to HDL_2 and HDL_3. The binding assay described in Experimental Procedures was used to determine the fraction of each protein that bound to HDL_2 and to HDL_3, and these values are normalized to the fraction (52 ± 1%) of human apoA-I bound to HDL_3. The apoA-I variants have been identified in the legends of Figures 1, 4, and 5. The white and hatched bars in panels A−C refer to binding to HDL_2 and HDL_3, respectively. (A) HDL_2 vs HDL_3 partitioning of human apoA-I and its N- and C-terminal domains. (B) Influence of the C-terminal segment and its hydrophobicity on binding of apoA-I to HDL_2 and HDL_3. (C) HDL_2 vs HDL_3 partitioning of human apoA-I and human−mouse hybrid molecules described in the legend of Figure 4. The fractional binding values are plotted as means ± SD ($n \geq 9$). For the data in all panels, 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.

Y236A) reduces the hydrophobicity[32] 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)[32] 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.[32]

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_2 and HDL_3. As shown in Figure 6A, when a small amount of [^{14}C]apoA-I is added to a mixture containing equal amounts of HDL_2 and HDL_3 (on a protein basis), human apoA-I and its N- and C-terminal domains bind more to HDL_3. In the case of the intact human apoA-I molecule, the level of binding to HDL_3 is approximately twice that to HDL_2. Removal of the C-terminal domain (variant 1−189) causes a larger reduction in the level of binding to HDL_2 than to HDL_3, so that the relative partitioning to HDL_3 is enhanced. A relative enhancement in binding to HDL_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

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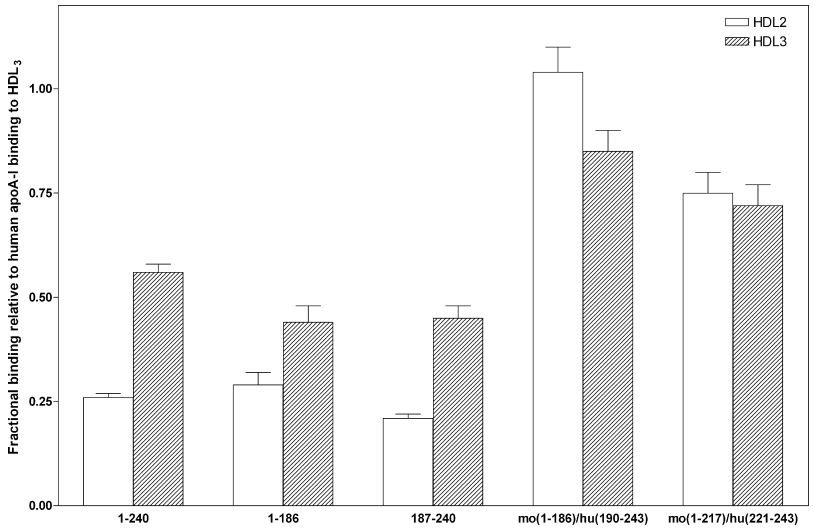

Figure 7. Relative abilities of mouse apoA-I variants to bind to HDL_2 and HDL_3. The experimental data were obtained and are presented as described in the legend of Figure 6. The white and hatched bars refer to binding to HDL_2 and HDL_3, respectively. The mouse apoA-I (white) and the N- and C-terminal 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 ($n \geq 6$). 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 HDL_3.

apoA-I to HDL_2 more than to HDL_3. Restoration of the C-terminal segment hydrophobicity (variant F225L/F229L/A232L/Y236L) restores the relative HDL_2−HDL_3 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 HDL_2 and HDL_3 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 HDL_2 and HDL_3, with the segment of the protein spanning residues 165−209 contributing to this effect.[35,57] We compared the HDL_2 versus HDL_3 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 HDL_2 versus HDL_3 partitioning. Under the conditions of our assay that contains equal amounts of HDL_2 and HDL_3, mouse apoA-I binds relatively well to HDL_3. This result is in contrast to an earlier finding that mouse apoA-I binds more to HDL_2;[35] the discrepancy is presumably a consequence of different experimental conditions. The isolated N- and C-terminal domains of mouse apoA-I partition more to HDL_3 than to HDL_2, with the relative preference for HDL_3 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 HDL_2 and HDL_3. The enhancement of apoA-I binding is greater with HDL_2, so that these hybrids partition approximately equally between HDL_2 and HDL_3 (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 HDL_2 and HDL_3.

Our prior SPR experiments[19] demonstrated that binding of apoA-I to HDL involves, in part, protein−protein interactions, and because HDL_3 has a higher ratio of apoA-II to apoA-I than HDL_2,[58,59] we tested the hypothesis that increased apoA-I−apoA-II interactions are responsible for the enhanced binding of apoA-I to HDL_3 (Figure 6). We separated the LpA-I and LpA-I with A-II fractions from HDL_2 and HDL_3 and compared the binding of apoA-I to each. The presence of apoA-II did not affect the partitioning of apoA-I between the HDL_2 and HDL_3 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 HDL_3 is not significantly affected and the level of binding to A-II-HDL_2 greatly reduced (Figure 8). It follows that the higher apoA-II content of HDL_3 relative to that of HDL_2 is not the reason for apoA-I binding better to HDL_3. Furthermore, the enrichment of the HDL_2 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 HDL_2 and HDL_3 are different, with the latter particle being more negatively charged.[46] To test the concept that the extra negative charge on HDL_3 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 HDL_2 and HDL_3 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)

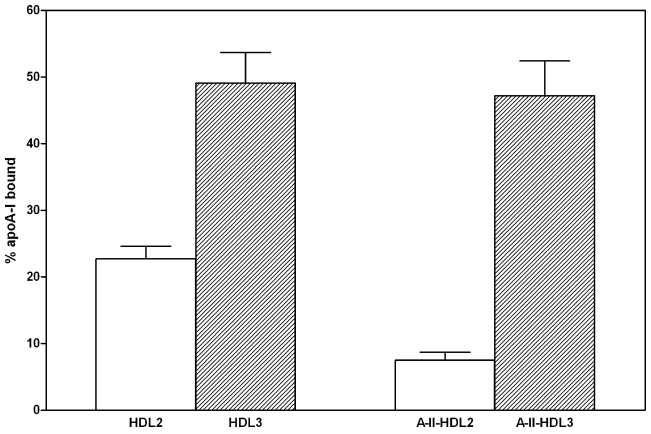

Figure 8. Effect of pretreatment with apoA-II on the binding of human apoA-I to HDL_2 and HDL_3. The characteristics of the apoA-II-pretreated HDL_2 and HDL_3 (A-II-HDL) are described in Experimental Procedures. The percent of [^{14}C]apoA-I bound to each HDL fraction was determined using mixtures containing either HDL_2 and HDL_3, A-II-HDL_2 with HDL_3, or HDL_2 with A-II-HDL_3. The amounts of bound apoA-I are plotted as means ± the standard error of the mean (SEM) (n = 3). The amounts of apoA-I bound to HDL_2 and A-II-HDL_2 are significantly different (p = 0.0001) by an unpaired t test, whereas the values for HDL_3 and A-II-HDL_3 are not significantly different.

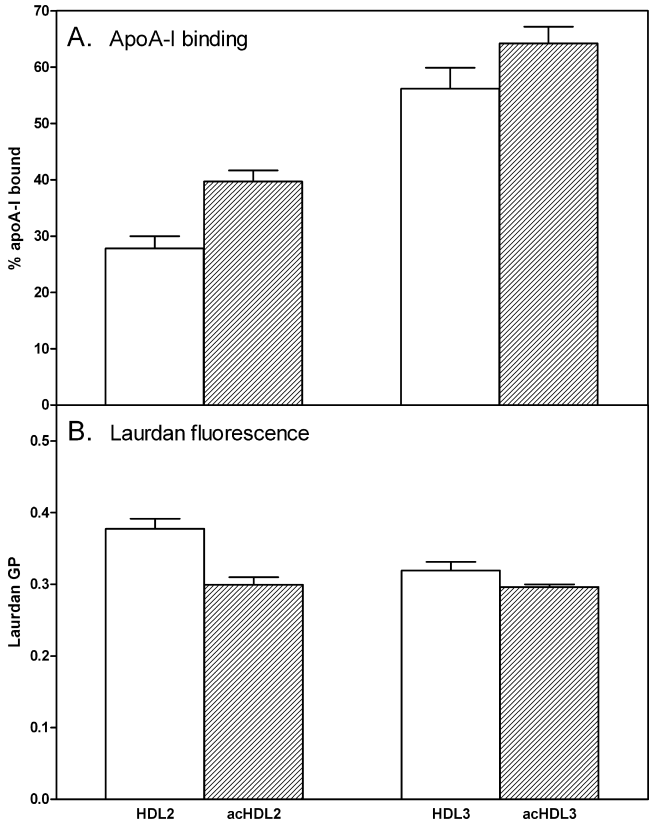

Figure 9. Influence of HDL surface charge and fluidity on apoA-I binding. (A) Effect of acetylation on the binding of human apoA-I to HDL_2 and HDL_3. The characteristics of acetylated HDL_2 ($acHDL_2$) and HDL_3 ($acHDL_3$) are described in Experimental Procedures. The percent of [^{14}C]apoA-I bound to each HDL fraction was determined using mixtures containing either HDL_2 and HDL_3, $acHDL_2$ and HDL_3, or HDL_2 and $acHDL_3$. The amounts of bound apoA-I are plotted as means ± SEM (n = 3). By an unpaired t test, the level of binding of apoA-I to $acHDL_2$ is significantly greater (p = 0.002) than to HDL_2 and the level of binding to $acHDL_3$ is significantly greater (p = 0.04) than to HDL_3. (B) Effect of acetylation on the PL order on the surfaces of HDL_2 and HDL_3 particles. The Laurdan generalized polarization (GP) was measured as described in Experimental Procedures, and the values are plotted as means ± SEM ($n \geq 7$). By an unpaired t test, the Laurdan GP in $acHDL_2$ is significantly lower (p = 0.0005) than that in HDL_2, whereas the values for $acHDL_3$ and HDL_3 are not significantly different ($p > 0.05$).

in the PL monolayer.[48,49] The Laurdan GP is lower in HDL_3 than in HDL_2 (Figure 9B), indicating that the PL order is lower in the former particle. The greater free space in the HDL_3 particle surface leads to enhanced apoA-I binding. Consistent with this idea, acetylation of HDL_2 and HDL_3 reduces the Laurdan GP, with the effect being larger for HDL_2 (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, namely that of mediating the initial interaction with the HDL surface.[19,60] This process 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.[19] Such interactions are responsible for the isolated mouse C-terminal domain, which is relatively polar[30] and has very limited ability to bind to a PL-stabilized emulsion (Figure 2B), binding to HDL essentially as well 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 HDL_3 than to HDL_2 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 HDL_2 and HDL_3 particles[61] that the total surface area of each type of particle in the mixture is similar (the total HDL_2 surface area is ~10% greater). It is of interest to understand the mechanisms responsible for the preferential binding of apoA-I to HDL_3. Estimates of the areas occupied by the PL and protein constituents present at the

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particle surface[42,62] indicate that ~30 and ~20%, respectively, of the surfaces of HDL_2 and HDL_3 particles are PL-covered. The greater availability of PL-covered surface in HDL_2 that can give rise to enhanced nonpolar apoA-I helix–PL interactions is presumably the reason that, relative to the HDL_3 case, binding to HDL_2 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 HDL_2 compared to HDL_3 (Figure 7) is most likely also due to hydrophobic apoA-I–PL interactions being more important for HDL_2 binding than for HDL_3 binding. Obviously, because the HDL_2 and HDL_3 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,[63] it is possible that the higher apoA-II content of HDL_3 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 HDL_2 and HDL_3 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 HDL_3 than to HDL_2, it is to be expected that PL molecular packing is looser on the HDL_3 particle surface. The Laurdan fluorescence polarization results (Figure 9) demonstrate the PL order is indeed lower in HDL_3 than in HDL_2, explaining why apoA-I binds relatively well to HDL_3. This finding is consistent with comparisons between different classes of lipoproteins showing that increased surface fluidity enhances the reactivity to lipolytic enzymes.[64] Why is the HDL_3 particle surface more disordered than that of HDL_2? The net negative surface charge is higher for HDL_3 particles,[46] 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 HDL_2. This change in PL molecular packing allows an increased level of apoA-I binding (Figure 9). Thus, HDL_3 is a better substrate than HDL_2 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,[9,42] but a labile pool of easily dissociable apoA-I molecules also exists.[14,15] The current observation that the surface structure of HDL_3 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.[15] This observation coupled with the fact that in normal human plasma the concentration of apoA-I in HDL_3 is greater than that in HDL_2[65] suggests that HDL_3 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.[2,6,16] 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[20,21] also interact similarly and become transported in plasma as part of HDL. There is evidence[21] that, compared to HDL_2, the HDL_3 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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