rapid communication

Apolipoprotein A-I conformation markedly influences HDL interaction with scavenger receptor BI

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Abstract Apolipoprotein A-I (apoA-I) is an important ligand for the high density lipoprotein (HDL) scavenger receptor class B type I (SR-BI). SR-BI binds both free and lipoprotein-associated apoA-I, but the effects of particle size, composition, and apolipoprotein conformation on HDL binding to SR-BI are not understood. We have studied the effect of apoA-I conformation on particle binding using native HDL and reconstituted HDL particles of defined composition and size. SR-BI expressed in transfected Chinese hamster ovary cells was shown to bind human HDL_2 with greater affinity than HDL_3, suggesting that HDL size, composition, and possibly apolipoprotein conformation influence HDL binding to SR-BI. To discriminate between these factors, SR-BI binding was studied further using reconstituted L-α-palmitoyloleoyl-phosphatidylcholine-containing HDL particles having identical components and equal amounts of apoA-I, but differing in size (7.8 vs. 9.6 nm in diameter) and apoA-I conformation. The affinity of binding to SR-BI was significantly greater (50-fold) for the larger (9.6-nm) particle than for the 7.8-nm particle. We conclude that differences in apoA-I conformation in different-sized particles markedly influence apoA-I recognition by SR-BI. Preferential binding of larger HDL particles to SR-BI would promote productive selective cholesteryl ester uptake from larger cholesteryl ester-rich HDL over lipid-poor HDL.—de Beer, M. C., D. M. Durbin, L. Cai, A. Jonas, F. C. de Beer, and D. R. van der Westhuyzen. **Apolipoprotein A-I conformation markedly influences HDL interaction with scavenger receptor BI.** ***J. Lipid Res.*** **2001.** 42: **309–313.**

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The scavenger receptor class B type I (SR-BI) plays an important role in high density lipoprotein (HDL) metabolism. SR-BI binds HDL and mediates selective lipid uptake into the liver and steroidogenic cells (1, 2). Selective lipid uptake is the mechanism whereby cholesteryl ester (CE) is taken up from HDL bound at the cell surface without internalization of the whole HDL particle (3–5). Interestingly, SR-BI exhibits a broad ligand specificity and binds low density lipoprotein (LDL), oxidized LDL, and very low density lipoprotein in addition to HDL (1, 6). The significance of SR-BI in the metabolism of non-HDL lipoproteins is not yet clear. Vesicles containing anionic phospholipids also bind to SR-BI, as do the apolipoproteins A-I, A-II, and C-III, either as lipoprotein-bound or as lipid-free proteins (7). HDL represents a mixture of particles that differ in size, composition, and apolipoprotein content, but the influence of these factors on the interaction of HDL with SR-BI is not known. As the major apolipoprotein of HDL, apolipoprotein A-I (apoA-I) represents a key ligand of SR-BI and a direct interaction of multiple sites in apoA-I with SR-BI has been shown by cross-linking (8). Current evidence suggests that amphipathic α helices of apolipoproteins are the recognition motif for SR-BI (7, 8). In this study we report that differences in apoA-I conformation markedly influence its recognition by SR-BI. Preferential binding of larger HDL particles to SR-BI is observed and would serve to promote selective CE uptake from larger lipid-rich HDL over lipid-poor HDL. Smaller HDL particles, such as SR-BI-generated remnants and preβ-HDL, would not be sequestered by SR-BI and could, instead, act as effective SR-BI-independent acceptors of cellular cholesterol.

MATERIALS AND METHODS

Ligands

Human HDL_2 (d = 1. 09–1.11 g/ml) and HDL_3 (d = 1.13–1.18 g/ml) were isolated by density gradient ultracentrifugation

Abbreviations: BSA, bovine serum albumin; CE, cholesteryl ester; CHO, Chinese hamster ovary; DPPC, dipalmitoyl-phosphatidylcholine; HDL, high density lipoprotein; LCAT, lecithin:cholesterol acyltransferase; POPC, L-α-palmitoyloleoyl-phosphatidylcholine; rHDL, reconstituted HDL; SR-BI, scavenger receptor class B type I.

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as previously described (9). Reconstituted HDL containing human apoA-I of particle size 96 Å (96-Å rHDL) was prepared by the sodium cholate dialysis method, using apoA-I/cholesterol/L-α-palmitoyloleoylphosphatidyl choline (POPC) molar ratios of 1:5:95 (10, 11). Smaller rHDLs, 78 Å in diameter (78-Å rHDL), were prepared with apoA-I/cholesterol/POPC molar ratios of 1:2:40. The 96-Å rHDL were essentially homogeneous (>98%), but the 78-Å rHDL required a two-step gel-filtration chromatography process to isolate pure 78-Å rHDL from a mixture of larger rHDL and lipid-free apoA-I produced in the rHDL preparation. First, the 78-Å rHDL and lipid-free apoA-I were isolated with a Superdex 200 column (1 × 30 cm) on a Pharmacia (Uppsala, Sweden) fast protein liquid chromatography system with a running buffer of 10 mM Tris, 150 mM NaCl, 1 mM NaN_3, 0.01% ethylenediaminetetraacetic acid, pH 8.0 (standard buffer). In the second step, the 78-Å rHDL were isolated from lipid-free apoA-I, using the same column equilibrated with the standard buffer plus 0.1 M guanidine hydrochloride. The column elution fractions containing 78-Å rHDL were immediately dialyzed against standard buffer to remove guanidine hydrochloride. The purity and size of rHDL were examined on 8–25% gradient gels under nondenaturing conditions, using the Pharmacia Phast system. Chemical cross-linking with bis(sulfosuccinimidyl)suberate (BS^3) was used to determine the number of molecules of apolipoprotein per particle. Experiments were performed within 10 days of particle preparation to avoid time-dependent size rearrangement of particles.

Human HDL was double-labeled with sodium [^{125}I]iodide, using the iodine monochloride method (12), and with [1α,2α(*n*)-^{3}H]cholesteryl oleoyl ether (13). The specific activity of the HDL ranged from 55 to 125 ^{125}I cpm/ng of protein and from 4 to 16 ^{3}H dpm/ng of protein. rHDL was labeled with ^{3}H by adding [^{3}H]dipalmitoyl-phosphatidylcholine ([^{3}H]DPPC) (2-palmitoyl-9,10-[^{3}H]; NEN Life Science Products, Boston, MA) (40–50 cpm/ng of apoA-I) to the lipids prior to preparation of the complexes. Alternatively, rHDL was iodinated with ^{125}I to a specific activity of 189–216 cpm/ng of protein for 78-Å rHDL and 461–690 cpm/ng of protein for 96-Å rHDL. Iodinated ligands were used within 48 h of labeling.

Ligand binding and uptake assays

Human SR-BI cDNA was amplified by polymerase chain reaction and cloned into the expression vector pCMV5 (14). Chinese hamster ovary (CHO)-ldlA7 cells stably transfected with human SR-BI (CHO-SRBI) were produced and maintained as previously described (9). Ligand binding to CHO-SRBI cells was carried out in 12-well plates essentially as described by Acton et al. (6). Cell association at 37°C was performed in Ham's F12 medium containing penicillin (100 units/ml), streptomycin (100 μg/ml), 2 mM glutamine, 0.5% fatty acid-free bovine serum albumin (BSA), and ^{125}I-labeled HDL. For binding at 4°C, cells were preincubated at 37°C for 1 h, washed with ice-cold 50 mM Tris-HCl, 150 mM NaCl, pH 7.4, containing 0.2% BSA, and then incubated at 4°C for 2 h with the indicated ligand in Ham's F12 buffered with 20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, pH 7.4, and containing 0.5% BSA. After incubation for the required time, cells were washed four times with 50 mM Tris-HCl (pH 7.4) containing 150 mM NaCl and 0.2% BSA, followed by two washes with 50 mM Tris-HCl, 150 mM NaCl (pH 7.4), and then dissolved in 0.1 N NaOH for radioactivity measurement and protein determination. Ligand degradation products were measured in the culture medium and in all cases were <15% of the cell-associated material. Apparent K_d values for binding were determined by nonlinear regression analysis of the SR-BI-specific cell associated values (total cell-associated values minus corresponding values for untransfected control cells), using Prism software (GraphPad, San Diego, CA).

RESULTS

To assess the possible effect of particle composition, size, and apolipoprotein conformation on the recognition of HDL by SR-BI, we compared the interaction at 37°C of human HDL_2 and HDL_3 with CHO cells transfected with human SR-BI (**Fig. 1**). SR-BI bound HDL_2 with a significantly greater affinity than HDL_3 (HDL_2, apparent K_d = 23 ± 3 μg/ml, HDL_3 apparent K_d = 44 ± 4 μg/ml; Fig. 1A). Values shown represent SR-BI-specific values that are calculated as the difference between the binding to CHO-SRBI cells and untransfected control ldl-A7 cells. In six separate experiments using three different preparations of HDL, the binding of HDL_2 was approximately 5-fold higher (range, 2.7- to 8.5-fold) than the binding of HDL_3 at a concentration of 10 μg/ml. The amounts of selective [^{3}H]CE uptake are shown in Fig. 1B. Selective uptake values were determined by subtracting the amount of surface-bound CE (calculated from the ^{125}I-labeled cell-associated values) from the total amount of CE associated with the cells, and represent the amount of CE internalized by cells in the absence of whole particle uptake. Selective uptake was greater from HDL_2 than HDL_3. The dif-

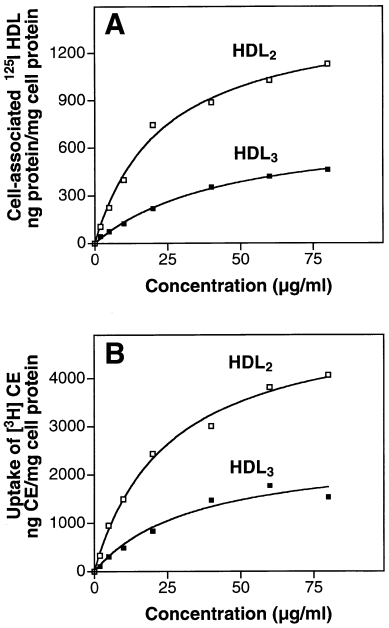

Fig. 1. Concentration-dependent association of HDL_2 and HDL_3 with CHO-SRBI cells. Cells were incubated for 2 h with the indicated concentrations of ^{125}I,^{3}H-labeled HDLs and the cell-associated label was quantitated as described in Materials and Methods. Shown are SR-BI-specific values, which were calculated as the difference between values for SR-BI-transfected cells and nontransfected CHO-ldlA7 cells. A: Cell-associated ^{125}I-labeled HDL_2 and HDL_3. B: Selective uptake of [^{3}H]CE from HDL_2 and HDL_3. Selective CE uptake was calculated by subtracting the amount of bound CE (calculated from the ^{125}I-labeled cell-associated radioactivity) from the total amount of cell-associated [^{3}H]CE. Values represent the mean of duplicate determinations.

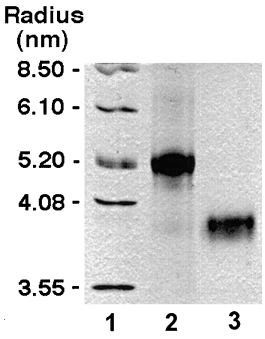

Fig. 2. Nondenaturing gradient gel electrophoresis of rHDL particles. rHDL particles were analyzed by 8–25% gradient gels and stained with Coomassie blue. Lane 1, molecular size markers with Stokes radii indicated; lane 2, 96-Å rHDL; lane 3, 78-Å rHDL.

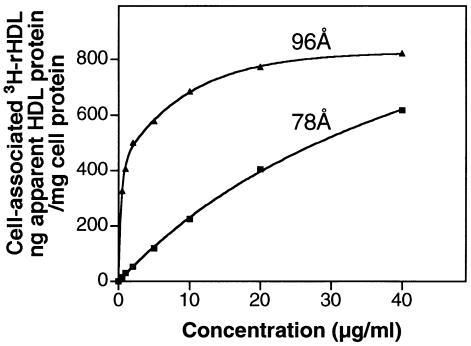

Fig. 4. Concentration-dependent association of ^{3}H-labeled rHDL with CHO-SRBI cells at 37°C. Cells were incubated for 30 min at 37°C with increasing concentrations of ^{3}H-labeled 78-Å and 96-Å rHDL. SR-BI-specific cell-associated label was quantitated as described in Fig. 1. Values represent the average of duplicate determinations.

ference between the HDL classes closely reflected the difference in SR-BI binding of the two ligands. The greater rates of SR-BI-mediated CE uptake from HDL_2 compared with HDL_3 are therefore largely due to differences in the affinity with which the ligands are bound by SR-BI.

To examine the possible influence of particle size and apolipoprotein conformation (in the absence of compositional differences) on the ability of apoA-I to bind to SR-BI, reconstituted apoA-I-containing lipoprotein discs of different size namely, 78 and 96 Å, were prepared. The 78-Å and 96-Å rHDL were each >90% homogeneous in size (**Fig. 2**). Chemical cross-linking with BS^3 was used to determine the number of apolipoprotein molecules per particle. Both types of particles contained two molecules of apoA-I as determined by cross-linking and <5% lipid-free apoA-I. The particles were tested for their ability to bind to CHO-SRBI cells. Binding of both rHDLs was rapid and reached maximum values within approximately 20 min (data not shown). SR-BI-specific binding of ^{125}I-labeled rHDL to CHO-SRBI cells at 37°C is shown in **Fig. 3**. The 96-Å rHDL bound to SR-BI with an approximately 50-fold greater affinity than the 78-Å rHDL. The apparent K_d values

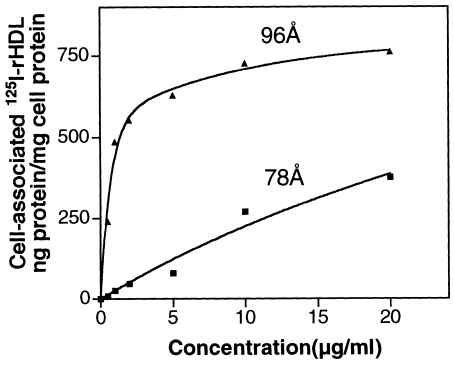

Fig. 3. Concentration-dependent binding of 78-Å and 96-Å rHDL to SR-BI-transfected CHO cells. Cells were incubated at 37°C for 30 min with increasing concentrations of ^{125}I-labeled 78-Å rHDL or 96-Å rHDL and the SR-BI-specific cell-associated label was quantitated as described in Fig. 1. Values represent the mean of duplicate determinations.

for 96-Å rHDL and 78-Å rHDL were 0.84 and 48 μg/ml, respectively. In three separate experiments the binding of rHDL at 5 μg/ml was between 7.6- and 8.2-fold greater for 96-Å rHDL than 78-Å rHDL. The binding affinity for 96-Å rHDL was relatively high, approximately 10- to 20-fold higher than that reported by us and others for plasma HDL, which was in the range of 15–30 μg/ml (6, 9, 15).

To confirm that the binding of ^{125}I-labeled apoA-I to SR-BI represents the binding of intact rHDL particles and not the binding of a lipid-free or lipid-poor apoA-I subfraction, or of apoA-I-enriched remnants that might be generated during the incubation of rHDL with CHO-SRBI cells at 37°C, rHDL was labeled in an alternative manner with ^{3}H in the phospholipid (PL) component. As shown in **Fig. 4**, [^{3}H]PL-labeled 96-Å rHDL (apparent K_d = 0.97 ± 0.2 μg/ml) also bound with markedly greater affinity than 78-Å rHDL (apparent K_d = 52 ± 3.3 μg/ml). The amount of rHDL binding, when calculated on a per particle basis, was similar for the ^{125}I-labeled and [^{3}H]PL-labeled rHDL (Figs. 3 and 4). These results indicate the binding to SR-BI of intact rHDL particles and not apoA-I-rich material. A marked difference in affinity of the two particles was also observed when binding of [^{3}H]PL-labeled rHDL was carried out at 4°C (data not shown). Maximum binding of [^{3}H]PL-labeled rHDL at 37°C was reached within 30 min and then remained at a constant level during a 2-h incubation period. Together, these results provide strong evidence that the cell-associated ^{3}H label in these experiments represents cell association of intact HDL-associated PL and not products generated from PL hydrolysis during incubation of rHDL with cells. Hydrolysis of PL in rHDL has been previously reported during incubation of rHDL with mouse Y1 cells (16). At 4°C, the [^{3}H]PL-labeled 96-Å rHDL (K_d = 2.5 ± 0.4 μg/ml) bound with approximately 10-fold greater affinity than 78-Å rHDL (K_d = 23 ± 8 μg/ml).

DISCUSSION

Two major findings are presented. First, HDL_2 and HDL_3 exhibit different binding affinities for SR-BI. HDL_2

has a higher binding affinity and a correspondingly greater rate of selective delivery of CE via SR-BI at subsaturating concentrations of HDL. Second, apoA-I-containing rHDL binding to SR-BI is markedly influenced by particle size and presumably apoA-I conformation. These results provide evidence that larger cholesteryl ester-rich HDL is the preferred ligand for SR-BI.

The greater affinity of HDL_2 binding to SR-BI compared with HDL_3 confirms the recent report by Liadaki et al. (15). In addition, we show in this study that greater binding of HDL_2 was accompanied by greater selective lipid uptake. If one considers the relative efficiency with which these two ligands deliver CE to cells once bound to SR-BI at the cell surface, and if efficiency of selective CE uptake is defined as uptake relative to the amount of surface bound ligand, then HDL_2 and HDL_3 have similar efficiencies of selective uptake. Thus, the greater rate of selective uptake from HDL_2 compared with HDL_3 at a given ligand concentration, is largely explained by greater SR-BI binding affinity for HDL_2 compared with HDL_3, and not to any significant difference in the ability of these two particles, once bound to SR-BI, to donate CE to SR-BI for receptor-mediated uptake. Previous studies reported that the rate of SR-BI mediated selective uptake from reconstituted lipoprotein particles was influenced by the amount of CE in such particles (16). Particles containing 1.3 mol% CE delivered 2.6 times more CE than particles containing only 0.6 mol%, indicating that the amount of this lipid in the core of the particle could become rate-limiting at the low levels found in these reconstituted discs. Our findings comparing HDL_2 and HDL_3 indicate that within the concentration range of CE found in HDL particles, the rate of SR-BI-mediated uptake is independent of CE content. In an earlier study, Pittman et al. (17) reported that denser HDL was slightly more efficient in selective uptake in adrenal Y1-BS1 cells than less dense HDL. The explanation for the difference between our results and this finding is not clear. Synthetic HDL and rat HDL were used in the earlier study and uptake was followed in Y1-BS1 cells. SR-BI-specific uptake was not measured in the Y1-BS1 cells, which also exhibited a much a greater proportion of nonselective, whole particle uptake than transfected CHO cells.

The marked differences in affinity of different-sized rHDL particles for SR-BI is evident for rHDL that contain the same number of apoA-I molecules (two molecules per particle), no other apolipoproteins, and identical lipid (POPC) component. These findings therefore indicate that conformational differences in apoA-I between the 78-Å rHDL and 96-Å rHDL particles exert a pronounced effect on the ability of apoA-I to act as a ligand for SR-BI. Such conformational change may account for the observed difference in binding affinities between HDL_2 and HDL_3. It may also explain the finding that preβ-1 HDL exhibits a low affinity for SR-BI (15). The ability of SR-BI to bind lipid-free apoA-I has been previously studied (8, 15). Direct binding assays showed high affinity binding of lipid-free apoA-I by SR-BI (8). On the other hand, chemical cross-linking showed clear differences between SR-BI binding of apoA-I in HDL and lipid-free apoA-I (8). A markedly reduced ability of lipid-free apoA-I to bind SR-BI was reported from competition analysis (15). The explanation for these discrepancies is not clear but may reflect differences in binding temperature (4 or 37°C), apoA-I oxidation, or lipid-free apoA-I aggregation.

The nature of the binding site on apoA-I for SR-BI is unknown. In the case of discoidal rHDL, apoA-I conformation is known to differ between the smaller 78-Å and larger 96-Å rHDL particles. The 96-Å rHDL have 72 ± 5% α-helix content compared with 59 ± 4% in 78-Å rHDL (18, 19). Fluorescence spectral properties of the two rHDL species differ as well. Although the wavelengths of maximal fluorescence of tryptophan residues are comparable, their accessibility to quenching is decreased in the 78-Å rHDL particles (18, 19). Also, the surface potential of both particles is distinct and the negative charge of the 78-Å rHDL is decreased by about 1.4 eV (19). In addition to the structural differences between the apoA-I in the two particles, the ability of apoA-I to activate lecithin:cholesterol acyltransferase (LCAT) is decreased by about 10-fold in the 78-Å rHDL (18, 20). Because the region of apoA-I involved in LCAT activation has been localized to residues 144–186 in its sequence (21), it is likely that the structural differences between the 96-Å rHDL and the 78-Å rHDL are also localized in this central region of apoA-I.

The analysis of apoA-I fragments has provided some interesting data on the nature of the SR-BI-binding site on apoA-I. Both the N-terminal (residues 1–86) and C-terminal (residues 148–243) domains of apoA-I were reported to bind independently to SR-BI in direct binding assays (8), with the C-terminal domain exhibiting a higher affinity. In addition, a model class A α helix was shown to bind SR-BI with high affinity (8). These results suggest that multiple amphipathic α-helical sites on apoA-I might bind to SR-BI. This is consistent with the finding that deletion of either the C terminus (residues 185–243) or N terminus (residues 1–59) from apoA-I did not abolish binding (15). Deletion of both these domains did diminish the affinity for SR-BI, although this effect is difficult to interpret because it might be caused by major conformational changes resulting from such large deletions (15). The C terminus is important for the initial association with lipids and for the formation of lipoprotein particles (21). Interestingly, while the C-terminal deletion mutant produced 77- to 79-Å discs that were smaller than the 96-Å discs prepared with full-length apoA-I, these discs exhibited near-normal binding to SR-BI (15). This suggests, by comparison with our results with 78-Å rHDL, that the size of particles, per se, does not influence the binding to SR-BI, rather that the conformation of apoA-I in the central region plays a key role in its binding to the receptor.

Our results demonstrate that the ability of lipid-associated apoA-I to act as a ligand for SR-BI is markedly dependent on HDL particle size and apoA-I conformation associated with the particle size. This feature may have important consequences for both selective lipid uptake and cellular cholesterol efflux. The greater binding affinity of larger particles, as shown in the case of HDL_2 com-

pared with HDL_3, results in greater CE uptake from HDL_2. Differences in binding affinities of different-sized HDL, probably related to conformational differences in apoA-I, therefore serve to maximize, through the preferential binding of larger cholesteryl ester-rich HDL, the rate of selective lipid uptake by SR-BI in vivo. The process of selective CE uptake from the core of HDL particles is expected to lead to a reduction in particle size. It is known that SR-BI can transfer phospholipids and cholesterol between HDL and cells, but uptake of apolipoprotein does not occur (16). Our findings indicate that the process of selective CE uptake from any given particle would tend to be a self-limiting process because the smaller remnant particles generated as a result of selective uptake would have a reduced affinity for SR-BI and would tend to be readily displaced by larger HDL particles, thereby increasing the efficiency of the SR-BI-mediated uptake process. The reduced binding affinity of smaller particles may also have significance for cellular cholesterol efflux. Small SR-BI-derived remnant particles or particles, such as preβ-HDL, which are known to be efficient acceptors of cholesterol, would bind SR-BI poorly and therefore not be sequestered by SR-BI, for instance, in the liver. This could facilitate the functioning of such acceptor particles in the efflux of cellular cholesterol by SR-BI-independent mechanisms.

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