Scavenger Receptor Class B Type I-mediated Cholesteryl Ester-selective Uptake and Efflux of Unesterified Cholesterol

INFLUENCE OF HIGH DENSITY LIPOPROTEIN SIZE AND STRUCTURE*

Received for publication, October 24, 2003, and in revised form, December 18, 2003
Published, JBC Papers in Press, January 12, 2004, DOI 10.1074/jbc.M311718200

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Scavenger receptor (SR)-BI catalyzes the selective uptake of cholesteryl ester (CE) from high density lipoprotein (HDL) by a two-step process that involves the following: 1) binding of HDL to the receptor and 2) diffusion of the CE molecules into the cell plasma membrane. We examined the effects of the size of discoidal HDL particles containing wild-type (WT) apoA-I on selective uptake of CE and efflux of cellular free (unesterified) cholesteryl (FC) from COS-7 cells expressing SR-BI to determine the following: 1) the influence of apoA-I conformation on the lipid transfer process, and 2) the contribution of receptor binding-dependent processes to the overall efflux of cellular FC. Large (10 nm diameter) reconstituted HDL bound to SR-BI better (Bmax ~420 versus 220 ng of apoA-I/mg cell protein), delivered more CE, and promoted more FC efflux than small (~8 nm) particles. When normalized to the number of reconstituted HDL particles bound to the receptor, the efficiencies of either CE uptake or FC efflux with these particles were the same indicating that altering the conformation of WT apoA-I modulates binding to the receptor (step 1) but does not change the efficiency of the subsequent lipid transfer (step 2); this implies that binding induces an optimal alignment of the WT apoA-I-SR-BI complex so that the efficiency of lipid transfer is always the same. FC efflux to HDL is affected both by binding of HDL to SR-BI and by the ability of the receptor to perturb the packing of FC molecules in the cell plasma membrane.

Understanding the metabolism of high density lipoprotein (HDL) is important in light of epidemiological studies demonstrating a positive correlation between high plasma levels of HDL and reduction in the incidence of atherosclerosis (1). Studies in recent years have identified the cell surface receptor, scavenger receptor (SR)-BI, as playing a key role in the cellular metabolism of HDL (for review see Refs. 2 and 3). Consequently, the interaction between HDL and SR-BI is under intense investigation. Early studies on SR-BI demonstrated that it can mediate the selective transfer of cholesteryl ester (CE) from HDL into cells (4) and also function to promote the bidirectional flux of free cholesterol (FC) between HDL and the cell plasma membrane (5). This functionality resides in the extracellular region of the receptor (6, 7). Despite intensive investigations, many aspects of the mechanism of SR-BI-mediated lipid flux (e.g. FC efflux and CE-selective uptake) between HDL and the cell plasma membrane remain unclear.

With respect to the selective uptake of CE, we (8, 9) and others (10) have proposed that SR-BI functions in a two-step process where the binding of the HDL to the receptor is coupled to the flux of lipids (such a reaction scheme is analyzed in the Appendix to Ref. 9). We have proposed that the lipid transfer step involves a hydrophobic pathway created between the bound HDL particle and the cell plasma membrane (8), whereas others have favored a hemifusion process (7). We have also shown that despite being of similar size and structure, rHDL composed of apoA-I and apoE have different CE-selective uptake efficiencies (11), implying that an appropriate alignment of the bound HDL and SR-BI is required for this process to occur optimally. This idea of proper ligand alignment on SR-BI and formation of a “productive complex” has been shown with a mutant of apoA-I that displays a reduced FC efflux capacity despite similar binding of HDL (12). Early studies using both synthetic rHDL and rat HDL showed that higher density (hence smaller diameter) HDL gives more selective CE uptake (13). However, by using Chinese hamster ovary cells expressing SR-BI, de Beer et al. (14) concluded that less dense, bigger diameter HDLs are able to deliver more CE compared with higher density HDLs. The fact that HDL size affects SR-BI-mediated selective CE uptake suggests that the nature of the HDL-SR-BI interaction is critical, although this is poorly understood at this time. It has been shown (14) that differences in apoA-I conformation in differently sized rHDL particles influence binding to SR-BI (step 1), but the consequences for the subsequent lipid flux (step 2) have not been investigated. Insight into how apoA-I conformation affects the lipid transfer will help in understanding the performances of different apoA-I-containing HDL particles (e.g. lipoprotein A-I) in reverse cholesterol transport (3).

Studies using cholesterol oxidase have indicated that SR-BI is capable of perturbing the plasma membrane in such a way that the enzyme has easier access to the FC (15), and FC efflux...
to acceptors such as cycloextrinsics is enhanced by this plasma membrane reorganization (16). This led to the idea that SR-BI does not have to interact directly with an extracellular acceptor of FC in order to promote FC efflux (17). However, a different line of evidence suggests that binding of an extracellular acceptor to SR-BI is necessary for FC efflux (18). Binding to simply hold the acceptor HDL particles near the plasma membrane is insufficient to enhance efflux (17). Thus, the quantitative contribution to FC efflux of binding of an extracellular FC acceptor such as HDL to SR-BI is not established, and the mechanism of SR-BI-mediated FC efflux remains controversial (cf. Refs. 12, 17, and 18).

To address the gaps in understanding of the mechanism of SR-BI-mediated lipid transfer outlined above, we studied the influence of the conformation of the wild-type (WT) apoA-I molecule on the facilitation of lipid flux via SR-BI, and we defined the contributions of the ligand binding and membrane perturbing properties of SR-BI to the efflux of cellular cholesterol to HDL. The results show that although WT apoA-I conformation influences the degree of HDL binding to SR-BI (step 1), the efficiency of the lipid transfer process (step 2) is independent of WT apoA-I conformation. In the case of SR-BI-mediated efflux of cellular FC, the lipid transfer step is more complicated because although binding of HDL to SR-BI affects FC efflux at low HDL concentrations, the membrane-perturbing effects of SR-BI contribute to changes in the efflux under conditions where binding to SR-BI is saturated.

**EXPERIMENTAL PROCEDURES**

**Materials**

1-Palmitoyl-2-oleoyl phosphatidylcholine (POPC) dissolved in chloroform was purchased from Avanti Polar Lipids (Alabaster, AL). Carrier-free Na\(^{23}\) (15 Ci/mmol) was purchased from PerkinElmer Life Sciences. L-[\(^{1,2-3}\)H]Cholesteryl oleyl ether (49 Ci/mmol) was purchased from Amersham Biosciences. Ham’s F-10, Dulbecco’s minimal essential media, and minimal essential media tissue culture media were purchased from BioWhittaker (Walkersville, MD). Fetal bovine serum was purchased from Sigma. FuGENE 6 was purchased from Roche Diagnostics. Ham\(^{1}\)/H11002 was purchased from BioWhittaker (Walkersville, MD). Etfal bovine serum was purchased from Sigma. PuGene 6 was purchased from Roche Diagnostics. Biacore, and minimal essential media tissue culture media were purchased from BioWhittaker (Walkersville, MD). Fetal bovine serum was purchased from Sigma. FuGENE 6 was purchased from Roche Diagnostics. Ham\(^{1}\)/H11002 was purchased from BioWhittaker (Walkersville, MD). Etfal bovine serum was purchased from Sigma. PuGene 6 was purchased from Roche Diagnostics. Biacore, and minimal essential media tissue culture media were purchased from BioWhittaker (Walkersville, MD). Fetal bovine serum was purchased from Sigma. FuGENE 6 was purchased from Roche Diagnostics.

**Normalplasmidic serum was used to isolate WT human apoA-I by using established methods (19). The generation of the expression plasmid (pET32) and point mutations of apoA-I have been described previously (20). Briefly, the human apoA-I cDNA was mutagenized by using the PCR with the following primers designed to introduce point mutations (20). Briefly, the human apoA-I cDNA was mutagenized by using the PCR with the following primers designed to introduce point mutations (20). Briefly, the human apoA-I cDNA was mutagenized by using the PCR with the following primers designed to introduce point mutations (20).

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**Methods**

**Discoidal Reconstituted HDL (rHDL) Particles—rHDL containing apoA-I and POPC was prepared by the cholate dispersion method (22). rHDL particles containing POPC and apoA-I at initial molar ratios of 100:1 or 40:1 were prepared. The resulting rHDL particles were passed through a Superdex 200HR gel filtration column (1 × 60 cm, Amersham Biosciences), and the appropriate fractions were isolated. The 125I-labeled apoA-I was then used in experiments to determine their FC efflux capacities. To determine the selective uptake CE from rHDL, apoA-I was first labeled with 125I by using the iodine monochloride method (23). This 125I-apoA-I was then complexed with POPC (at 100:1 or 40:1 mol/mol) containing 1 mol % of cholesteryl oleate and a trace amount of [\(^{3}\)H]cholesteryl oleyl ether (specific activity of 24 cpm/ng cholesteryl oleate) using the cholate dispersion method (22). 100:1 mol/mol POPC/apoA-I rHDL containing D102A/D103A apoA-I was also prepared in parallel. The latter was isolated and characterized as described above. Both WT and D102A/D103A apoA-I generated similar 10 nm diameter rHDL particles. The phospholipid content of the particles was determined enzymatically using phospholipase D (Wako Chemical, Richmond, VA). His\(_{6}\)alosuccinimidylsulfoxamate (BS\(_{3}\)) (24) was used to chemically cross-link the apoA-I molecules within the rHDL particles to assess the number of apoA-I molecules per particle. Lipid-free apoA-I, large rHDL, and small rHDL were incubated with 10 mg/ml BS\(_{3}\) in 0.1 M phosphate buffer for 3.5 h, and the reaction was quenched with 250 mg ethanol-
amine. The samples were then fractionated using 8–25% gradient SDS-PAGE, and the degree of apoA-I oligomerization was determined.

Cell Culture Experiments—COS-7 kidney cells that had been transiently transfected with SR-BI were used in all experiments. The cells were maintained in Dulbecco’s minimal essential media supplemented with 10% calf serum, penicillin (50 units/ml), and streptomycin (0.1 mg/ml). Plasmid cDNA containing SR-BI or plasmid cDNA alone was transiently transfected in this cell line with FuGENE 6 as the carrier by using an established procedure (6). To determine the cellular efflux of cholesterol, the transfected cells were labeled with [3H]cholesterol, and efflux was measured as described previously (17). The binding of dual-labeled rHDL and the selective uptake of CE, defined as the amount of CE uptake in excess of that due to whole particle uptake, were determined as described (8).

RESULTS

Generation of rHDL Particles of Two Different Sizes—To examine the effects of HDL size on its ability to bind to SR-BI, promote selective CE uptake, and serve as an acceptor of FC, we generated two different sized rHDLs composed of apoA-I and POPC. By using established methods, rHDL of ~8 and 10 nm in diameter were created (Fig. 1A). As assessed by chemical cross-linking (Fig. 1B), these rHDLs both contained an average of two apoA-I molecules per particle. In addition, they had similar phospholipid to CE ratios (in the range 25–30 mol/mol) with the major difference between the two particles being the phospholipid to protein ratio. The final phospholipid to protein mass ratio for the large (~10 nm particle) rHDL was 1.7 ± 0.2 (70:1 mol/mol) and for the small (~8 nm particle) rHDL it was 0.6 ± 0.06 (30:1 mol/mol); these parameters are similar to those reported previously by others (25).

Large rHDLs Bind More Than Small rHDLs to SR-BI and Deliver More CE to Cells—To compare the functionalities of small and large rHDL, we incubated dual-labeled ([3H]cholesteryl oleyl ether and 125I-apoA-I) rHDL particles for 2 h at 37 °C in minimal essential media. The binding of large (■) or small (▲) rHDL (A) and selective uptake of CE for large (▲) and small (○) rHDL (B) were determined as described previously (8). C, the binding data from A are expressed as the amount of PL bound using the phospholipid to protein mass ratios of 1.7 and 0.6 for the large and small rHDL, respectively. D shows the binding data expressed as the concentration of rHDL phospholipid in the incubation medium. Data points are SR-BI-specific values obtained after subtracting the values from control (mock-transfected) cells and represent the mean of triplicate wells ± S.E. The data shown are from one representative experiment of three independent experiments using three preparations of rHDL.

![Figure 2](image-url)

**Fig. 2.** Large rHDLs bind better than small rHDLs to SR-BI and allow more CE-selective uptake. COS-7 cells cultured as described under “Methods” were transiently transfected with either the vector containing SR-BI cDNA or vector DNA alone and incubated with the indicated concentrations of dual-labeled ([3H]cholesteryl oleyl ether and 125I-apoA-I) rHDL particles for 2 h at 37 °C in minimal essential media. The binding of large (■) or small (▲) rHDL (A) and selective uptake of CE for large (▲) and small (○) rHDL (B) were determined as described previously (8). C, the binding data from A are expressed as the amount of PL bound using the phospholipid to protein mass ratios of 1.7 and 0.6 for the large and small rHDL, respectively. D shows the binding data expressed as the concentration of rHDL phospholipid in the incubation medium. Data points are SR-BI-specific values obtained after subtracting the values from control (mock-transfected) cells and represent the mean of triplicate wells ± S.E. The data shown are from one representative experiment of three independent experiments using three preparations of rHDL.
hamster ovary cells transiently expressing SR-BI (14), but they show a similar effect of HDL size. Because both types of rHDL contained two apoA-I molecules per particle (Fig. 1B), the concentration expressed as μg of apoA-I/ml is indicative of the particle number (i.e., at a given protein concentration, the same number of each size of rHDL particles was present). When the data are expressed as μg of phospholipid/ml of the ligand present in the medium (Fig. 2D), the difference in the binding and selective uptake between the two sizes of rHDL remains. Note that if the binding of the ligand is expressed as the amount of PL bound, the difference in the amount of binding between the large and small rHDL particles is even more dramatic (Fig. 2C). Correspondingly, the amount of CE-selective uptake was dependent on the amount of rHDL binding (Fig. 2B). The absolute amount of CE-selective uptake was diminished for the small rHDL, but when normalized to the amount of rHDL bound to SR-BI, both sizes showed the same efficiency of CE-selective uptake (Fig. 3). In Fig. 3A, both the binding and selective CE uptake are plotted on the same axes, and it is evident that both parameters are correlated. In Fig. 3B, the binding of rHDL expressed as the number of rHDL particles bound is plotted against the selective uptake of CE; the correlations for both sizes of rHDL are fitted by linear regression (R² values of 0.93). The slopes of both lines are similar indicating that despite differences in the total amount of ligand bound, bound rHDL particles of both sizes are equally efficient in delivering CE.

**Large rHDL Is a Better Acceptor of Cellular FC Than Small rHDL**—To examine if the greater binding of large rHDL leads to enhanced efflux of FC, COS-7 cells were labeled with [3H]FC, and the efflux capacities of large and small rHDL were compared. Fig. 4A shows that larger rHDL induced more FC efflux than small rHDL when compared on the basis of particle (protein) concentration. The results in Fig. 4A are for FC efflux after 2 h of incubation, but the difference observed between the large and small rHDL remained for incubations ranging from 30 min to 6 h (data not shown). There was still a difference if the FC efflux was measured as a function of the amount of rHDL phospholipid present in the incubation medium (Fig. 4B). In Fig. 4C, the concentration dependence of HDL binding and FC efflux is compared. It is apparent that after the binding of both sizes of rHDL particles had saturated, the efflux of FC continued to rise. This is clearly seen in Fig. 4D where FC efflux is plotted as a function of the number of rHDL particles bound to SR-BI. In the low range of HDL binding, the slopes of the lines for both the large and small HDLs are similar reflecting binding-dependent FC efflux of similar efficiency. However, once the receptors are saturated, the lines become approximately vertical in each case indicating that, despite saturation of binding sites on SR-BI, FC efflux continues to increase.

The Effect of ApoA-I Mutation on rHDL Binding, FC Efflux, and Selective CE Uptake—To test if the binding of HDL to SR-BI is in itself sufficient for optimal selective CE uptake, we examined the ability of apoA-I D102A/D103A to mediate this process. This mutant of apoA-I has been identified by its ability to bind, in an rHDL particle, to SR-BI similarly to the WT apoA-I but induce relatively low FC efflux (12). Substitution of the acidic residues in WT apoA-I with two non-polar residues imparted some differences in the physical parameters of the apolipoprotein. The most striking difference between the WT apoA-I and the D102A/D103A mutant was the lowered solubility of the mutant in aqueous buffer. In addition, compared with the WT protein, the lipid-free mutant was slightly less stable than the WT protein to denaturation, and more of its hydrophobic surface was exposed to the aqueous phase assessed by binding of 8-sulfinilamido-1-naphthalenesulfonic acid to the protein.² Despite these differences, D102A/D103A apoA-I formed large rHDLs of comparable size to WT apoA-I. Fig. 5A shows the concentration dependence of binding for these two rHDL particles. As reported by Liu et al. (12), the binding isotherms for rHDL containing WT apoA-I and apoA-I D102A/D103A are similar, although not identical. By using four independent preparations of rHDL, it appeared that the total amount of binding of the mutant rHDL was slightly more than that of the WT rHDL. Despite this similarity in binding, the mutant protein had a diminished capacity to promote FC efflux compared with the WT protein (Fig. 5B), as observed by Liu et al. (12). We also compared the effects of this apoA-I mutation on CE-selective uptake; similar to the reduction in FC efflux, the amount of CE-selective uptake was also reduced compared with the WT rHDL (Fig. 5C). To compare the effect of the apoA-I mutation on the functionality of the rHDL particles, we plotted the binding of the rHDL versus the selective uptake of CE (Fig. 5D). The steeper slope for the WT rHDL relative to that of the mutant rHDL indicates that the efficiency for CE-selective uptake was reduced due to the mutation in apoA-I (cf. Fig. 3B for large and small rHDL containing WT apoA-I where the

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² H. Saito, S. Lund-Katz, and M. C. Phillips, unpublished observation.
efficiencies were similar). A similar type of analysis to that of Fig. 5 for the FC efflux data showed that the efficiency of FC efflux was also reduced due to the apoA-I mutation (data not shown).

The Effects of Mutations in SR-BI on Binding of Discoidal rHDL and Selective CE Uptake—In addition to binding HDL, SR-BI has been shown to reorganize the FC in the cell membrane in such a way that cholesterol oxidase has easier access to the sterol (15). There are mutations in the receptor that cause it to lose this ability to increase the cholesterol oxidase-sensitive pool of membrane FC. For example, an SR-BI variant (tagVI) containing an epitope tag insertion toward the C-terminal end of the extracellular domain of the receptor can bind HDL, but it does not increase the cholesterol oxidase-sensitive pool of plasma membrane FC (26). Introduction of the point mutation G420H gives rise to the same phenotype.3 We used these two SR-BI variants to examine the possibility that the reduced binding of small rHDL compared with large rHDL (Fig. 2A) is due to the fact that, unlike large rHDL which binds directly to the receptor by apoA-I/SR-BI interaction (27), the small rHDL interacts only with the perturbed membrane lipid domain created by SR-BI rather than directly with the receptor itself. The observation that small rHDL bound equally well to WT SR-BI and the G420H variant (Fig. 6A) indicated that the perturbed membrane lipid domain did not contribute to binding of HDL. Most important, although the G420H mutation in SR-BI did not affect the binding of small rHDL, it significantly reduced the level of selective CE uptake (Fig. 6A). This SR-BI mutation reduced binding of large rHDL as well as selective CE uptake from this particle (Fig. 6B). The tag VI mutation in SR-BI decreased the binding of both small and large rHDL particles by ∼75% with concomitant decreases in CE-selective uptake (Fig. 6).

DISCUSSION

It is generally agreed that the mechanism of SR-BI-mediated lipid movement into a cell involves an initial binding step to form a “productive” receptor-ligand complex followed by a second step in which lipid molecules transfer from the bound donor particle into the cell plasma membrane. Experiments using SR-BI molecules with mutations in the extracellular domain have shown that the binding of HDL (step 1) and CE transfer to the cell plasma membrane (step 2) are correlated, although the two steps are independent. Thus, there are SR-BI mutants that bind HDL normally but exhibit defective CE-selective uptake (2). To address some of the molecular details and quantitative aspects of how the nature of the receptor-ligand complex modulates lipid transfer, we used discoidal rHDLs that have defined sizes, a constant CE/phospholipid ratio but different WT apoA-I conformations, and we compared CE uptake from these particles. In addition, we addressed the issue of the respective contributions to FC efflux of HDL bind-

3 M. A. Connelly and D. L. Williams, unpublished observations.
The D102A/D103A mutation in apoA-I reduces the ability of rHDL to promote SR-BI-mediated CE-selective uptake and FC efflux. A, COS-7 cells transiently transfected with either the vector containing SR-BI cDNA or vector DNA alone were incubated with the indicated concentrations of WT apoA-I rHDL (●) or D102A/D103A apoA-I rHDL (○) for 2 h at 37 °C. The rHDL were both ~10 nm in diameter. B, COS-7 cells expressing SR-BI prelabeled with [3H]FC were incubated with the indicated concentrations of WT rHDL (●) or D102A/D103A rHDL (○) for 2 h at 37 °C, and the efflux of [3H]FC was measured. C, COS-7 cells were incubated with the indicated concentrations of WT rHDL (●) or D102A/D103A rHDL (○) for 2 h at 37 °C, and the selective uptake of CE was determined. D, the binding of the WT rHDL (●) or D102A/D103A apoA-I rHDL (○) is plotted against the selective CE uptake. The $R^2$ values for the linear regression were 0.93 and 0.65 for WT and D102A/D103A apoA-I rHDL, respectively. rHDL binding, FC efflux, and CE-selective uptake values are the mean of values from duplicate wells (which did not vary by more than 10%) after values from control (mock-transfected) cells were subtracted. A representative data set is shown for each case; the experiments were repeated with three different preparations of rHDL for FC efflux and four different preparations of rHDL for the selective CE uptake.

Fig. 5. The D102A/D103A mutation in apoA-I reduces the ability of rHDL to promote SR-BI-mediated CE-selective uptake and FC efflux. A, COS-7 cells transiently transfected with either the vector containing SR-BI cDNA or vector DNA alone were incubated with the indicated concentrations of WT apoA-I rHDL (●) or D102A/D103A apoA-I rHDL (○) for 2 h at 37 °C. The rHDL were both ~10 nm in diameter. B, COS-7 cells expressing SR-BI prelabeled with [3H]FC were incubated with the indicated concentrations of WT rHDL (●) or D102A/D103A rHDL (○) for 2 h at 37 °C, and the efflux of [3H]FC was measured. C, COS-7 cells were incubated with the indicated concentrations of WT rHDL (●) or D102A/D103A rHDL (○) for 2 h at 37 °C, and the selective uptake of CE was determined. D, the binding of the WT rHDL (●) or D102A/D103A apoA-I rHDL (○) is plotted against the selective CE uptake. The $R^2$ values for the linear regression were 0.93 and 0.65 for WT and D102A/D103A apoA-I rHDL, respectively. rHDL binding, FC efflux, and CE-selective uptake values are the mean of values from duplicate wells (which did not vary by more than 10%) after values from control (mock-transfected) cells were subtracted. A representative data set is shown for each case; the experiments were repeated with three different preparations of rHDL for FC efflux and four different preparations of rHDL for the selective CE uptake.

ing to SR-BI and of the ability of the receptor to reorganize the packing of FC molecules in the cell plasma membrane.

CE-selective Uptake—It has been shown before that larger and less dense HDL bind better to SR-BI (14), and it was concluded that apoA-I conformation is a key determinant of this effect. We have reproduced this observation and extended the findings to include the consequence of this difference in binding on the subsequent lipid uptake. Because the rHDLs we used contain the same number of apoA-I molecules per particle, at a given apoA-I concentration the same number of large and small rHDL particles was present. Therefore, the difference in binding of the two sizes of rHDL (Fig. 2A) was not simply due to variations in the number of particles available for binding to the receptor. As noted before (14), the difference between the binding affinity of the large and small rHDL is most likely due to differences in the conformation of the apoA-I molecules on the two types of rHDL (the packing density of the two apoA-I molecules located around the edge of the larger discoidal rHDL particle is relatively low). This argument can also be used to explain the higher $B_{\text{max}}$ exhibited by the large rHDL (Fig. 2A); for instance, it is possible that there is an SR-BI-mediated self-association of the large but not the small rHDL particles at the cell surface. The difference in binding was paralleled by a similar trend in the selective uptake of CE (Fig. 2B); the larger rHDL gave rise to more selective uptake than the smaller rHDL. On closer examination of the data in Figs. 2 and 3, it is apparent that, despite an impairment of step 1, both sizes of rHDL particle were equally efficient at step 2. Thus, the slopes of the lines for both sizes of rHDL particle are identical in Fig. 3B indicating that, once the amount of binding to SR-BI is normalized, there is no impairment in the movement of CE into the cell. This is expected because the phospholipid to CE ratios were the same in the two sizes of rHDL particle, and it is the concentration gradient of CE between the bound HDL and the cell plasma membrane that drives the selective uptake process (cf. Ref. 8). This result implies that after WT apoA-I in different size HDL particles binds to SR-BI, it can be induced to give an optimal alignment so that the efficiency of the CE transfer step from HDL is the same.

The observation that differences in CE-selective uptake from HDL particles containing WT apoA-I were normalized, once variations in their binding to SR-BI and CE content were taken into account, does not apply generally. Thus, as seen in Fig. 5A, rHDL created with D102A/D103A apoA-I bound to SR-BI like those created with WT apoA-I, yet there was impaired movement of CE into the cell (Fig. 5C). Furthermore, the slopes of the lines relating rHDL binding to CE-selective uptake for the WT and mutant apoA-I rHDL particles are different (Fig. 5D). The lower slope for the mutant apoA-I rHDL confirms that selective uptake was less efficient for the mutant apoA-I particle. A possible explanation for this is that the putative hydrophobic channel between the bound HDL and the plasma membrane created by SR-BI (8) requires an appropriate organization of the bound HDL and the SR-BI. It seems that there are multiple binding sites on SR-BI for apoA-I (9) and, perhaps, one of these binding sites is energetically more favorable in allowing selective CE uptake. The model then suggests that rHDL created with WT apoA-I can be accommodated well at this site, whereas particles containing D102A/D103A apoA-I cannot. Apparently, introducing mutations into the apoA-I molecule and altering the primary structure is effectively equivalent to switching to another apolipoprotein.
Thus, the efficiency of lipid transfer is different for different apolipoproteins (11, 13), perhaps because alterations in the amino acid sequence of the ligand lead to binding at non-optimal sites. A detailed description of the tertiary and quaternary structure of SR-BI in the plasma membrane with and without bound apoA-I is needed to explore these ideas more fully.

Both sizes of the rHDL particle seem to bind directly with the receptor via an apoA-I/SR-BI interaction and not to a region of the plasma membrane where lipids are reorganized by SR-BI. Support for this concept comes from the fact that both types of SR-BI mutant described in Fig. 6 did not increase the cholesterol oxidase-sensitive pool of plasma membrane FC, yet they were both able to bind rHDL. Moreover, not only does the ligand have to situate itself properly on the receptor for efficient CE-selective uptake to occur, it appears that the receptor itself has to be in a proper conformation because we observed that a point mutation of SR-BI (G420H) bound the small rHDL similarly to WT SR-BI but showed a diminished selective CE uptake (Fig. 6A). In the case of the large rHDL, both the binding and efficiency of CE-selective uptake were reduced (Fig. 6B) suggesting that both the apoA-I interaction site and the hydrophobic channel are perturbed by the point mutation in SR-BI. Similarly, the alteration in SR-BI structure induced by the tag VI mutation affected both the apoA-I/SR-BI interaction (step 1) and the CE transfer (step 2).

FC Efflux—In addition to mediating selective CE uptake, SR-BI can promote the efflux of FC to an extracellular acceptor such as HDL. Based on the evidence presented here, it seems that the same mechanism that allows the selective uptake of CE to occur is at work for SR-BI-mediated FC efflux. There is evidence in support of binding-dependent (18) and binding-independent (17) SR-BI-mediated FC efflux to HDL. The current study suggests that both models are applicable depending upon the concentration of HDL ligand present in the extracellular medium. As reported by Gu et al. (18), FC efflux is binding-dependent at low concentrations of HDL where binding to SR-BI is not saturated. The facilitation of efflux in this condition is due perhaps to transfer of FC molecules from the plasma membrane to the bound HDL particle via a hydrophobic channel created by the SR-BI molecule (cf. Ref. 8). At saturating concentrations of HDL, FC efflux is binding-independent. In this condition, FC efflux presumably occurs by the aqueous diffusion mechanism (16) with the facilitation being due to enhanced desorption of FC molecules from the cholesterol oxidase-sensitive pool of plasma membrane FC created by the membrane-perturbing effects of SR-BI (15). Similar to CE-selective uptake, SR-BI-mediated FC efflux was size-dependent in that, at the same particle concentration, large rHDL promoted more FC efflux than small rHDL (Fig. 4A). Because the phospholipid content of HDL affects its ability to promote FC efflux (28), the enhanced efflux with large rHDL could be due to the greater phospholipid content of the large rHDL particles. There was a contribution from this effect because
expression of large and small rHDL concentrations in terms of phospholipid reduced the difference in efflux between the two types of particle (cf. Fig. 4, A and B). However, the difference in FC efflux was not eliminated by this normalization and the additional FC efflux to large rHDL was presumably due to the relatively high binding of the large rHDL particles (Fig. 4C).

The level of FC efflux was dependent on the number of rHDL particles bound to SR-BI at low ligand concentrations (below the point in Fig. 4D where the lines become approximately vertical), but once the receptor was saturated, the FC efflux became independent of rHDL binding to SR-BI.

Conclusions—Overall, the current study demonstrates that the two-step SR-BI-mediated flux of lipids between HDL and the cell membrane depends on the proper organization of both the bound ligand and the receptor. In contrast to the effects of mutations of either apoA-I or SR-BI that can alter both HDL/SR-BI binding (step 1) and the subsequent lipid transfer (step 2), alteration of the conformation of WT apoA-I in the HDL ligand affects only step 1. This result implies that when WT apoA-I binds to SR-BI, it can be induced to give an optimal alignment so that the efficiency of the lipid transfer step is the same. Thus, in vivo, the ability of different WT apoA-I-containing HDL particles to deliver CE to cells is probably controlled by their affinity for SR-BI. The critical organization of the HDL-SR-BI complex is the same in all cases so that the CE flux is controlled simply by the concentration gradient of CE between the bound HDL particle and the plasma membrane. The effects of WT apoA-I conformation (as altered by discoidal rHDL size) are the same for CE-selective uptake and FC efflux at low HDL concentrations, below receptor saturation, where efflux is primarily to bound HDL particles. However, efflux is also dependent on HDL concentration at higher concentrations where binding to SR-BI is saturated; under this condition, the ability of SR-BI to reorganize FC molecular packing in the cell plasma membrane contributes to the change in FC efflux.

Acknowledgments—We thank Faye Baldwin and Denise Schrader for expert technical assistance.

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