Characterization of Two High-Density Lipoprotein Binding Sites on Porcine Hepatocyte Plasma Membranes: Contribution of Scavenger Receptor Class B Type I (SR-BI) to the Low-Affinity Component†

Laurent O. Martinez, Valérie Georgeaud, Corinne Rolland, Xavier Collet, François Tercé, Bertrand Perret, and Ronald Barbaras*

Institut Féderatif de Recherche d'immunologie moleculaire et cellulaire, IFR 30, Institut National de la Santé et de la Recherche Médicale, Unité 326, Phospholipides membranaires, Signalisation cellulaire et Lipoprotéines, Hôpital Purpan, 31059, Toulouse Cedex, France

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ABSTRACT: Two HDL_3 high- and low-affinity binding sites are present on the human hepatoma cell line ($HepG_2$). Recently, we have suggested that the high-affinity binding sites might modulate the endocytosis of HDL through the low-affinity binding sites [Guendouzi, K. (1998) *Biochemistry 37,* 14974−14980], highlighting the physiological importance of this family of HDL high-affinity binding sites. The present data demonstrate the presence of HDL_3 high-affinity (K_d = 0.37 μg/mL, B_{max} = 260 ng/mg of protein) and low-affinity (K_d = 86.2 μg/mL, B_{max} = 14 300 ng/mg of protein) binding sites on purified porcine hepatocyte plasma membranes. By contrast, free apoA-I was strictly specific to the high-affinity sites (K_d = 0.2 μg/mL and B_{max} = 72 ng/mg of protein). Competition experiments between ^{125}I-labeled HDL_3 and either LDL, oxidized LDL, or anti-SR-BI IgG as competitors show that SR-BI is mostly responsible (70% displacement) for the binding of HDL_3 to the low-affinity binding sites. By contrast, the same competition experiments using ^{125}I-labeled free apoA-I clearly excluded SR-BI as the high-affinity binding receptor. We conclude that the binding of HDL onto hepatocyte plasma membranes involves: (1) two low-affinity binding receptors, one being SR-BI; (2) one family of high-affinity binding sites unrelated to SR-BI.

Several high-density lipoprotein (HDL)[1] binding proteins have recently been cloned, and their role in HDL metabolism is currently being assessed. Today much is known about the scavenger receptor BI (SR-BI), a member of the class B scavenger receptors mainly described in rodents, which presents 80% homology with the human CLA-1 receptor (CD36 LIMP II analogous protein 1) (*1*−*3*). The level of SR-BI expression correlates with the selective uptake of cholesteryl ester into steroidogenic cells (*4*) and with cholesterol efflux from peripheral cells (*5*, *6*). These events might occur after docking of HDL at the cell surface, but the mechanism of this HDL−SR-BI interaction still remains unknown. One major difficulty is that SR-BI presents a broad ligand specificity (i.e., LDL, oxLDL, acetylated LDL, anionic phospholipids, and HDL). Furthermore, the potential contribution of other membrane proteins modulating the SR-BI-mediated cholesteryl ester uptake in hepatocytes was never excluded (*7*). Previous studies on rat membrane hepatocytes and human hepatocyte cell lines ($HepG_2$) have shown the existence of two families of HDL_3 binding sites (*8*, *9*). The HDL_3 low-affinity binding sites on $HepG_2$ cells induce the endocytosis of HDL_3 through the formation of clathrin-coated vesicles, a mechanism which apparently does not involve SR-BI (*10*−*12*). However, we cannot totally exclude that proteins different from SR-BI, i.e., CLA1, could be involved in the low-affinity binding. Recently, we have suggested that engagement of the high-affinity binding sites could trigger the endocytosis of HDL through the low-affinity binding sites on $HepG_2$ cells (*13*). To better understand the potential importance of this HDL high-affinity family of binding sites, we attempted to define more precisely the binding of HDL and free apoA-I on native pig hepatocytes and on purified pig hepatocyte plasma membranes. The pig (*Sus scrofa domesticus*) model presents analogies with humans in terms of lipoprotein metabolism (*14*), and the use of the pig liver for xenotransplantation experiments in humans requires a better understanding of the interactions between HDL and the pig hepatocytes (*15*). In addition to the convenience of using plasma membrane preparations (large amounts available), this material allows exclusion of any contribution of the cellular metabolism to the binding studies. In this work, we demonstrate, first, the existence, on purified pig hepatocyte plasma membranes, of two families of HDL binding sites, implicating apoA-I with high-

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* Correspondence should be addressed to this author at Institut National de la Santé et de la Recherche Médicale, Unité 326, Phospholipides membranaires, Signalisation cellulaire et Lipoprotéines, Hôpital Purpan, 31059, Toulouse, France. Tel.: 33 561 77 94 14. Fax: 33 561 77 94 01. E-mail: Ronald.Barbaras@purpan.inserm.fr.

[1] Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; oxLDL, oxidized LDL; SR-BI, scavenger receptor class B type I; CLA-1, CD36 and LIMPII analogous protein 1; apo, apolipoprotein; SDS, sodium dodecyl sulfate; PBS, phosphate-buffered saline; BSA, bovine serum albumin.

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and low-affinity sites and, second, we show that SR-BI is not involved in the high-affinity binding sites but partly accounts for the low-affinity binding sites of HDL.

EXPERIMENTAL PROCEDURES

Materials. Dextran T500 and ^{125}I Na were obtained from Amersham Pharmacia Biotech (France). All cell culture reagents were from Life Technologies (France). Bovine serum albumin (BSA) and all other reagents (analytical grade) were from Sigma (La Verpilliere, France).

Lipoprotein, Apolipoprotein, and Proteoliposome Preparations. LDL and HDL_3 were isolated from the plasma of normolipidemic donors as previously described (*9*). LDL were oxidized by exposure for 3 h to 5 μM $CuSO_4$ at 37 °C (*16*). ApoA-I was isolated from HDL_3 by ion-exchange chromatography as described (*17*). The purity of apoA-I was checked by Western blot analyses (*18*) using different antibodies directed against human apoB, apoA-II, apo-C's, and apoA-I. The apolipoprotein A-I homogeneity was more than 99% (as measured by densitometry after SDS−PAGE and silver staining).

Complexes containing apoA-I and dimyristoylphosphatidylcholine (DMPC) were prepared by the cholate dialysis procedure (*19*) at a phosphatidylcholine:protein molar ratio of 150:1. The Stokes radius of the complexes was estimated to be 100 ± 10 Å (*19*).

^{125}I-labeling of free apoA-I and HDL_3 was performed by the *N*-bromosuccinimide method (*20*). Specific radioactivities for HDL ranged from 3000 to 5000 cpm/ng of protein. In the case of ^{125}I-apoA-I complexes, the apoA-I was labeled before insertion into the lipid complexes. The same preparation of ^{125}I-apoA-I was used either in a free form or complexed to DMPC in the binding experiments. The specific radioactivities ranged from 10 000 to 20 000 cpm/ng of apoA-I. More than 97% of the radioactivity was associated with protein.

Preparation of Porcine Plasma Membranes. Plasma membranes were prepared by the aqueous two-phases partition procedure as described by Navas and Morre (*21*). Potential contaminations by other subcellular compartments were detected by measurement of specific enzymatic markers (5′-nucleotidase for plasma membranes, galactosyltransferase for the Golgi apparatus, glucose-6-phosphatase for the endoplasmic reticulum, monoamine oxidase for mitochondria) as previously described (*10*).

Isolation of Porcine Hepatocytes. Hepatocytes were obtained from pig livers, using a slight modification of the two-step in situ collagenase perfusion method (*22*). Indeed, collagenase H (Boehringer Mannheim, Mannheim, Germany) instead of collagenase D was used for pig hepatocyte dissociation (*15*). Cells were maintained in William's medium, supplemented with 10% fetal calf serum, and were used within 2 days after dissociation.

SR-BI Antiserum Preparation. Three synthetic peptides were designed using rodent SR-BI and CLA-1 sequences (KGSQDKEAYQAYSES, LKGEKPVVRERGPYVYRE-FRHK, LGLNPDPREHSM). A mixture of these peptides was then injected to rabbits. Peptide synthesis and immunserum preparations were realized by Neosystem (Strasbourg, France). IgGs were purified using the ammonium sulfate precipitation method (*23*). The IgG homogeneity was more than 99%. Dot blot experiments confirm the binding of the SR-BI/CLA1 antibody to the peptides.

Western Blots. Proteins from pig hepatocyte plasma membranes were separated by 10% SDS−PAGE under nonreducing conditions and were transferred to a nitrocellulose membrane using a Bio-Rad transblot unit (30 min, 5.5 mA/cm^2). SR-BI was immunodetected with antipeptide antibodies (10 μg/mL) and revealed with a horseradish peroxidase-conjugated second antibody. The proteins were visualized by enhanced chemiluminescence (ECL) detection (Santa Cruz Biotechnology, Santa Cruz, CA).

Binding Assays. Binding of labeled lipoproteins and proteoliposomes to hepatocytes or purified hepatocyte plasma membranes was performed as previously described (*24*). Briefly, cell monolayers (300 000 cells/well) or 7 μg of plasma membrane proteins were incubated in PBS for 2 h at 4 °C in the presence of increasing concentrations of labeled ligands, and then treated as follows: (1) cells were washed twice with ice-cold PBS (maximum washing time was 15 s) and lysed with 500 μL of 0.1 N NaOH, and the NaOH digest was used for radioactivity measurement and protein determination; (2) plasma membranes were filtered on 0.22 μm filters (GVWP Millipore, France) and washed 3 times with 1% BSA in PBS as previously described (*8*). Filters were used for radioactivity measurements. Nonspecific binding was determined in the presence of a 100-fold excess (as compared to the K_d value) of the corresponding unlabeled ligand. The value of nonspecific binding varied from 25 to 30% of total binding. The ratio of bound to free ^{125}I-apolipoprotein versus bound ^{125}I-apolipoprotein was plotted according to Scatchard (*25*).

Kinetic Assays. The association rate constant (k_{+1}) of the different ligands was determined by measuring the amount of protein specifically bound on hepatocyte plasma membranes at 4 °C and at various times. The concentration of the radioactive ligand was constant during all the experiments. Nonspecific binding was determined with a 100-fold excess of the corresponding nonradioactive ligand. All the washing and radioactivity measurements were performed as for the binding assays. The pseudo-first-order method was used for k_{+1} calculation: considering L as the concentration of labeled ligand and B_e as the equilibrium binding value, the slope of $\ln[B_e/(B_e - B)]$ versus time gives the observed association rate constant (k_{ob}) (*26*, *27*). Determination of the dissociation rate constant (k_{-1}) from independent dissociation experiments (see below) allows calculation of $k_{+1} = (k_{ob} - k_{-1})/L$.

Dissociation experiments were carried out on hepatocyte plasma membranes at 4 °C, using a single concentration of labeled ligand. After the association equilibrium binding was reached (i.e., 100 min), a 100-fold excess of unlabeled ligand was added. Under these conditions and using a first-order equation, k_{-1} is calculated as the slope of $\ln(B/B_0)$ versus time (where B_0 is the binding value at time = 0, i.e., at equilibrium). Alternatively, k_{-1} can be estimated as $k_{-1} = 0.693/t_{1/2}$, where $t_{1/2}$ is the time when $B = 0.5B_0$ (*28*).

Competition Assays. As for the binding assay to plasma membranes, the competition experiments were performed at 4 °C for 2 h. Plasma membranes (7 μg of proteins) were incubated with a constant concentration of labeled ligand (5 μg/mL for HDL_3 and 1 μg/mL for free apoA-I), and increasing concentrations of unlabeled competitors were

Table 1: Marker Enzyme Characteristics of Plasma Membranes Isolated by Aqueous Two-Phase Partition[a]

	5′-nucleotidase (plasma membrane)	glucose-6-phosphatase (endoplasmic reticulum)	galactosyl transferase (Golgi apparatus)	monoamine oxidase (mitochondria)
homogenate	4.5 ± 1 (1)	7 ± 1.5 (1)	4.2 ± 0.5 (1)	2.7 ± 0.6 (1)
plasma membrane	105 ± 17 (23.3)	1.5 ± 0.3 (0.2)	1.3 ± 0.3 (0.3)	0.7 ± 0.2 (0.3)

[a] The units of specific activity are micromoles per hour per milligram of protein. Values in parentheses represent relative specific activities (enrichment factors). Values are representative of three independent dosages performed on three different plasma membrane preparations.

added. Washing and radioactivity measurements were performed as above. Data were expressed as the percentage of the specific binding measured in the absence of competitor versus the log of competitor concentration (in micrograms of protein per milliliter).

Data Analysis. Binding and competition data were analyzed using a weighted nonlinear curve-fitting program, based on the LIGAND analysis program (*29*).

Analytical Procedures. The protein concentration was determined by the method of Bradford (*30*), using the Bio-Rad protein assay dye and BSA, as a standard.

RESULTS

Purification of Plasma Membranes from Pig Liver. Starting from 500 g of pig liver, the aqueous two-phase partition procedure allowed us to obtain 50 mg of pure plasma membranes in less than 6 h. According to previous reports (*21*), the dominant orientation was right-side-out (cytoplasmic side in). The purity of plasma membrane preparations was tested by the measurement of specific enzyme markers for subcellular compartments (Table 1): the enrichment factor in plasma membranes marker was 23 while it was <0.3 for other markers, indicating the lack of contamination in agreement with previous results (*21, 31*). The possibility of contaminations by other cell types (mainly by endothelial cell types) was ruled out by Western blot analysis using an antibody directed against CD 36 receptor, a typical protein of these cells (data not shown). These results confirmed that our starting material was pure hepatocyte plasma membranes.

Characterization of HDL_3 and ApoA-I Binding on Hepatocyte Plasma Membranes. We have first determined the binding characteristics of HDL_3 on purified plasma membranes (Figure 1A) or isolated pig hepatocytes (Figure 1B). As previously described for human hepatoma cells ($HepG_2$) (*9*), we observed in both cases the presence of two binding components: one of low-affinity and high-capacity and the other of high-affinity and low-capacity as determined by the calculation of K_d and B_{max} values (Table 2). The higher B_{max} measured on plasma membrane preparations reflects the enrichment in plasma membrane markers. Thus, the plasma membrane purification procedure does not affect the binding characteristics of HDL_3.

Similar binding isotherms were obtained with apoA-I−DMPC complexes (Figure 1C), and the calculated binding parameters (Table 2) confirm the essential role of apoA-I in the binding of HDL_3 to pig hepatocyte plasma membranes. Finally, free apoA-I (not associated with lipids) displayed only high-affinity binding on plasma membranes (Figure 1D and Table 2).

Kinetic experiments of HDL_3 binding (Figure 2) were performed using 90 μg/mL ^{125}I-labeled HDL_3, and the measured parameters at this concentration correspond to the contribution of both the high- and low-affinity binding sites (Table 2). From the association curve (Figure 2A) and dissociation curve (Figure 2B), we determined the respective constants allowing calculation of the K_d value (Table 3). This value (84.4 μg/mL) was very comparable to the K_d of the low-affinity binding sites obtained from isotherm binding experiments (86.2 μg/mL, Table 2).

Table 2: Binding Parameters of ^{125}I-Labeled HDL_3, ^{125}I-Labeled ApoA-I−DMPC, and ^{125}I-Labeled Free ApoA-I to Hepatocytes and Purified Pig Hepatocyte Plasma Membranes at 4 °C[a]

	high affinity		low affinity	
	K_d (μg/mL)	B_{max} (ng/mg)	K_d (μg/mL)	B_{max} (ng/mg)
hepatocytes				
HDL_3	0.17	30	38.3	1285
membranes				
HDL_3	0.37	260	86.2	14300
apoA-I−DMPC	0.24	175	67.4	10300
free apoA-I	0.24	72		

[a] Data are obtained from a Scatchard plot of Figure 1 and are representative of three independent experiments on three different plasma membranes or two different isolated hepatocyte preparations.

Table 3: Kinetic Association and Dissociation Parameters

	HDL_3
k_{-1} (min^{-1})	0.0217
k_{+1} [min^{-1}·(μg/mL)$^{-1}$]	$2.57\ 10^{-4}$
K_d[a] (μg/mL)	84.44

[a] Calculated $K_d = k_{-1}/k_{+1}$.

Competition Experiments. We performed competition experiments using 5 μg/mL ^{125}I-labeled HDL_3 (Figure 3). Again, at this concentration, the contribution of both the high- and low-affinity binding sites is analyzed, but we measure essentially the low-affinity binding since the high-affinity component represents only 10% of the total binding observed. The competition obtained with apoA-I−DMPC complexes was identical to the displacement obtained with unlabeled HDL_3, indicating that apoA-I−DMPC complexes bind to the same family of binding sites as HDL_3 (Figure 3A). To test the contribution of SR-BI to the low-affinity binding sites, competition experiments were done between ^{125}I-labeled HDL_3, and LDL or oxLDL as competitors (Figure 3A). The results showed that SR-BI is mostly responsible for the binding of HDL_3 to the low-affinity binding sites since LDL and oxLDL competed to 70% with HDL_3. To confirm the contribution of SR-BI to the low-affinity binding sites, we prepared anti SR-BI/CLA-1 antibodies that recognize SR-BI protein (82 kDa) in different preparations of pig hepatocyte plasma membranes as well as in control CHO cells (Figure 4). The antibody could also detect a 2−3-fold increase in the protein in CHO cells overexpressing the SR-BI (kindly provided by Pr. A. Tall, New York) (*32*). The specificity of the SR-BI/CLA1 antibody was finally confirmed by the net decrease in the signal observed by Western blot analysis of plasma membranes in the presence of an

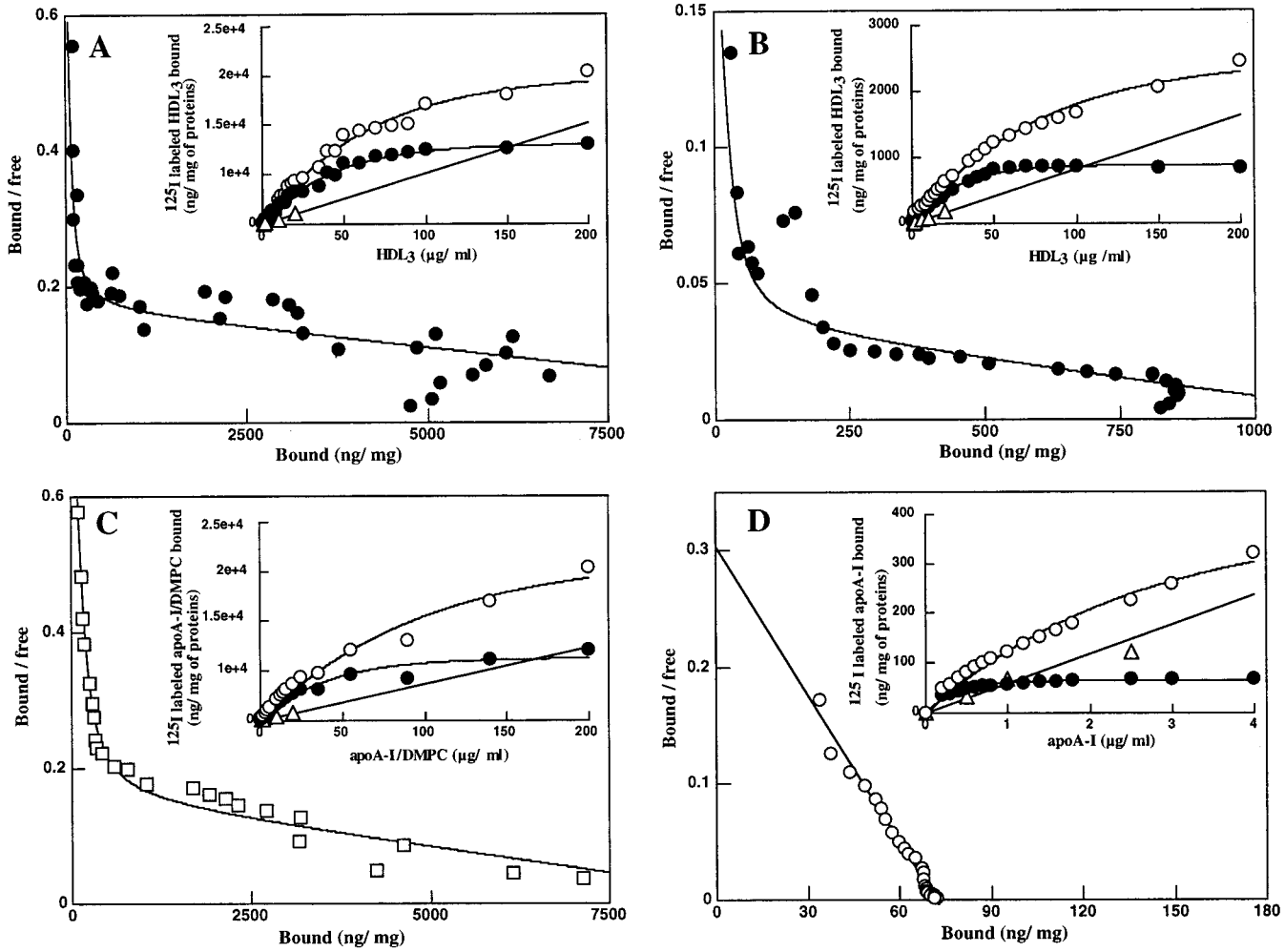

FIGURE 1: Scatchard representation and binding isotherm of ^{125}I-labeled HDL_3, ^{125}I-labeled apoA-I−DMPC, and ^{125}I-labeled free apoA-I to porcine hepatocyte plasma membranes or isolated hepatocytes. Specific ^{125}I-labeled HDL_3 (A, B), ^{125}I-labeled apoA-I−DMPC (C), or ^{125}I-labeled free apoA-I (D) binding was measured after 2 h incubation at 4 °C on plasma membranes (A, C, D) or isolated hepatocytes (B). Nonspecific binding was assessed as described under Experimental Procedures and represented 30% of the total binding. The solid line represents the computer-generated line of the best fit. The results are representative of three independent experiments performed on three different plasma membranes or two different isolated hepatocyte preparations. Inset: Corresponding binding isotherm of ^{125}I-labeled HDL_3 (A, B), ^{125}I-labeled apoA-I−DMPC (C), ^{125}I-labeled free apoA-I (D): total binding (○), specific binding (●), nonspecific binding (△).

excess of the three peptides used for antibody preparation (not shown). Although, some unreproducible minor bands could be detected in the different preparations, the antibody recognized mainly the SR-BI protein at 82 kDa. Purified anti SR-BI/CLA-1 IgG inhibited by 70% the binding of HDL_3 whereas no competition was observed with the preimmune serum IgG (Figure 3B).

To better characterize the high-affinity binding sites, we further performed competition experiments using ^{125}I-labeled free apoA-I, which specifically binds to these high-affinity binding sites on plasma membranes. Similar competition curves were obtained for free apoA-I, HDL_3, and apoA-I−DMPC complexes (Figure 5A), indicating that this high-affinity binding site was the same for the three ligands. By contrast, the lack of competition between ^{125}I-labeled free apoA-I and LDL, oxLDL (Figure 5A), or SR-BI/CLA-1 IgG (Figure 5B) clearly ruled out SR-BI from the family of HDL high-affinity binding sites.

DISCUSSION

The present study shows that porcine hepatocytes display high- and low-affinity binding sites for HDL_3 and apoA-I−DMPC complexes. Moreover, free apoA-I, without association with lipids, was able to bind only to the high-affinity binding sites. These data obtained on normal hepatocytes confirm earlier observations on the hepatoma-derived $HepG_2$ cell line (*9*). Furthermore, purified hepatocyte plasma membranes showed the same binding components and characteristics as intact hepatocytes, demonstrating that both families of HDL binding sites are present on the plasma membranes and are not affected by the isolation procedure.

It is very important to remember that at the low concentrations of free apoA-I that were used (<200 μg/mL), no apoA-I aggregation occurs, as previously reported by Stone and Reynolds (*33*), and that all the experiments were carried out at 4 °C in order to impede any protein and/or lipid exchanges. Trypsin treatment and heat shock (60 °C, 1 h) abolish the HDL_3 binding capacity of plasma membranes, strongly suggesting a protein−protein interaction (data not shown).

The affinity rate between the high- and low-affinity binding sites is around 230 times (Table 2), and this affinity is in the 10^{-7}−10^{-9} M range, considering a HDL_3 molecular mass of 200 kDa. Two other possibilities could be compatible with a concave-upward Scatchard plot observed for HDL_3

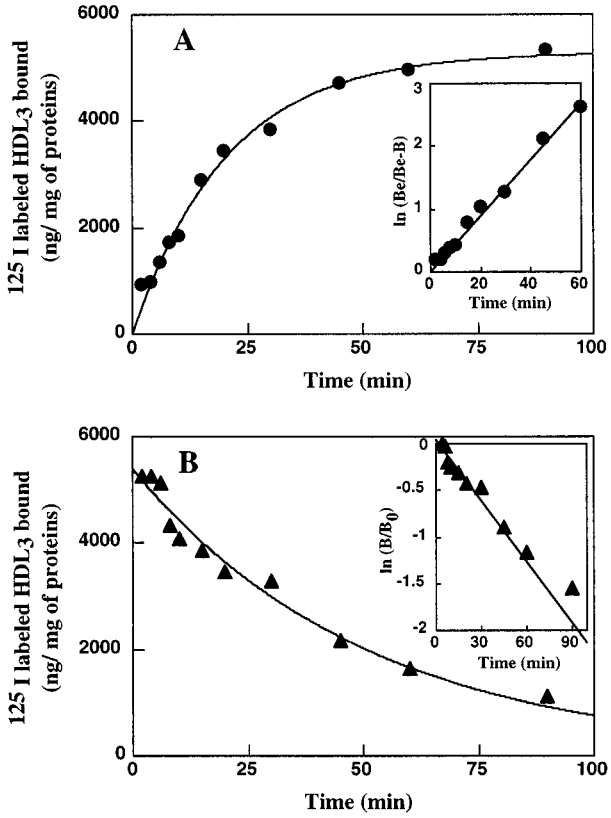

FIGURE 2: Kinetics of specific association (A) and specific dissociation (B) at 4 °C of ^{125}I-labeled HDL_3 onto porcine hepatocyte plasma membranes. A concentration of 90 μg/mL ^{125}I-labeled HDL_3 was used for specific association and specific dissociation experiments as a function of the time. A pseudo-first-order equation was used to calculate the association rate constant (k_{+1}) from the slope of ln [B_e/(B_e − B)] versus time (A, inset). After 90 min, unlabeled HDL_3 (9 mg/mL) was added for dissociation experiments (B). The dissociation rate constant (k_{-1}) was calculated from the slope of the plot of ln(B/B_0) versus time (B, inset). The curves represent the specific binding calculated as described under Experimental Procedures. Nonspecific binding averaged 30% of the total binding. The curves are representative of two independent experiments performed on two different plasma membrane preparations.

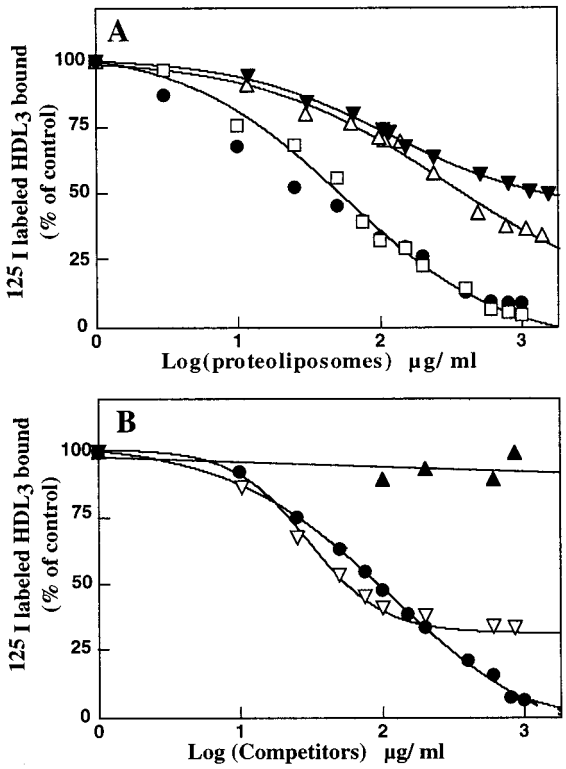

FIGURE 3: Competitive inhibition of the binding of ^{125}I-labeled HDL_3 to porcine hepatocyte plasma membranes. Porcine hepatocyte plasma membranes were incubated for 2 h at 4 °C in the presence of 5 μg/ml ^{125}I-labeled HDL_3 and increasing concentrations of (A): unlabeled HDL_3 (●), apoA-I−DMPC complexes (□), LDL (▼), and oxidized LDL (△); and (B): unlabeled HDL_3 (●), purified anti-SR-BI/CLA-1 IgG (▽), and preimmune serum IgG (▲). 100% specific binding corresponds to 1300 ng/mg of plasma membrane proteins. Nonspecific binding represent 25% of total binding. The results are representative of three independent experiments performed on three different plasma membrane preparations.

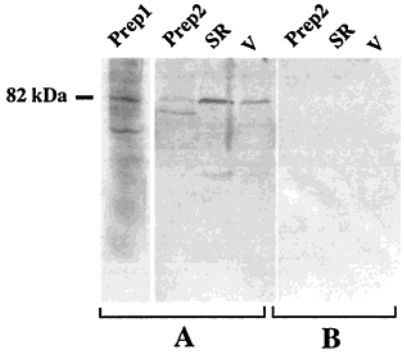

FIGURE 4: Western blot analysis. Plasma membrane proteins (20 μg) from two different pig hepatocyte preparations (Prep1 and Prep2) or from CHO cells transfected with vector only (V) or overexpressing SR-BI (SR) were immunoblotted with SR-BI specific IgG (A) or preimmune serum (B).

and apoA-I−DMPC complexes binding, namely, a negative cooperativity between the two families of sites or a lattice effect. However, the calculated K_d obtained from kinetic experiments was very comparable to the K_d value measured from Scatchard experiments, which would be different in the case of a negative cooperativity. Furthermore, free apoA-I high-affinity binding parameters were comparable to those obtained from HDL_3 binding experiments, suggesting that the free apoA-I and HDL_3 high-affinity binding sites were identical and that these high-affinity binding sites are not related to the low-affinity ones. Altogether these observations allow us to exclude a negative cooperativity. The lattice effect is a very complex event to be evaluated, and so far, we cannot totally rule out this possibility to explain the Scatchard plot. Nevertheless, if we compare the parameters of LDL binding for which a lattice effect was observed (*34*, *35*), we find differences in the HDL_3 characteristics: (i) K_d and B_{max} values of HDL_3 binding between the two families of sites are too different (230- and 55-fold respectively) to be due to a lattice effect only. (ii) The HDL_3 association is clearly saturable, so that no delay is needed for a repositioning of the bound ligand to maximize the accessibility of more binding sites for further binding as suggested by Chappell in the case of LDL (*34*, *35*). (iii) The dissociation experiment fits (Figure 2B) were monoexponential, suggesting a simple bimolecular binding.

The calculated K_d values derived from the association and dissociation rate constants were in the same range as the K_d calculated from the Scatchard analysis, confirming that the binding sites characterized by saturation experiments follow the law of mass action, indicative of a ligand−receptor interaction. Thus, we can conclude that the HDL_3 present

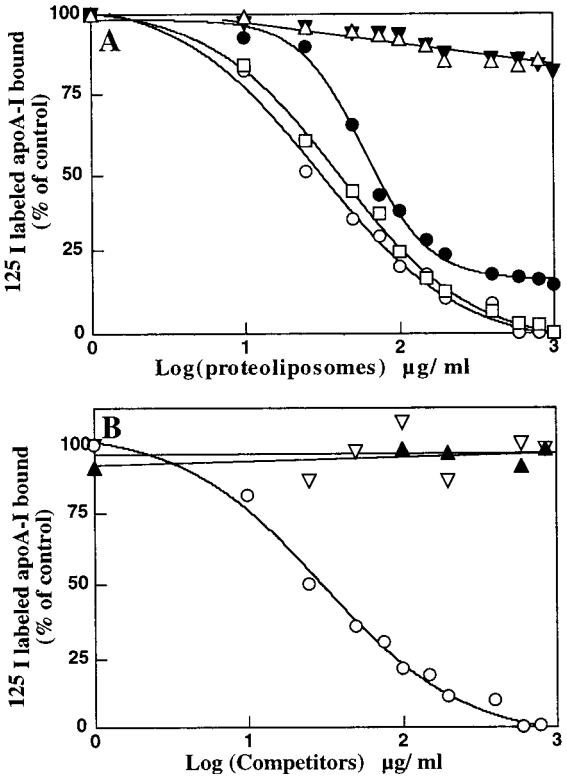

FIGURE 5: Competitive inhibition of the binding of ^{125}I-labeled free apoA-I to porcine hepatocyte plasma membranes. Porcine hepatocyte plasma membranes were incubated for 2 h at 4 °C in the presence of 1 μg/mL ^{125}I-labeled apoA-I and increasing concentrations of (A): unlabeled apoA-I (○), HDL_3 (●), apoA-I−DMPC complexes (□), LDL (▼), and oxidized LDL (△); and (B): unlabeled apoA-I (○), purified anti-SR-BI/CLA-1 IgG (▽), and preimmune serum IgG (▲). 100% specific binding corresponds to 130 ng/mg of plasma membrane proteins. Nonspecific binding represent 25% of total binding. The results are representative of three independent experiments performed on three different plasma membrane preparations.

high- and low-affinity independent binding sites on pig hepatocytes and that apoA-I is able to bind either the same two affinity binding sites when associated with lipids or only the high-affinity binding sites when apoA-I is not associated with lipid complexes.

SR-BI is the most extensively studied HDL receptor, and we attempted to identify the contribution of this receptor in HDL_3 binding on hepatocytes plasma membranes. Competition experiments with different ligands of SR-BI (HDL, LDL, oxLDL) or with an antibody designed against three peptides from SR-BI/CLA-1 indicate that SR-BI represents around 70% of the low-affinity binding sites. These observations are in good agreement with previous data showing the presence of HDL low-affinity binding sites involving SR-BI (*36*, *37*). Altogether, these results clearly show that SR-BI is mainly but not exclusively implicated in the low-affinity binding sites.

More interestingly, the lack of competition observed between free apoA-I and LDL, oxLDL, or SR-BI/CLA-1 IgG clearly ruled out SR-BI from the high-family of binding sites. Furthermore, free apoA-I and HDL_3 binding experiments using SR-BI-transfected CHO cells (*6*) (kindly provided by Pr. A. Tall) have shown the absence of HDL high-affinity binding sites on these cells (data not shown), confirming that the high-affinity binding sites were strictly independent of SR-BI.

The role of the high-affinity binding sites remains to be established. Nevertheless, we can suggest that, as for $HepG_2$ cells, they could modulate the functionality of either SR-BI or other unidentified low-affinity but high-capacity receptor. In addition, free apoA-I and lipid-poor apoA-I (pre β1 HDL) have been described in the plasma (*38*) and may act as a privileged regulator of the HDL catabolism through the high-affinity binding sites. Finally, the identification of this high-affinity binding site needs to be further established, and the model of purified porcine plasma membranes that we have characterized may prove useful for the isolation of this receptor, since the high-affinity binding for apoA-I was clearly maintained in the preparations.

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