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Topology of Scavenger Receptor Class B Type I (SR-BI) on Brush Border Membrane

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Abstract

Both hydropathy plot and *in vitro* translation results predict the topology of SR-BI; the receptor is an integral membrane protein of 509 amino acids, consisting of a short cytoplasmic N-terminus of 9 amino acids followed by a first transmembrane domain of 22 amino acids, the extracellular domain of 408 amino acids, the second transmembrane domain of 22 amino acids, and the cytoplasmic C-terminus of 47 amino acids. The immunoblot of rBBMV in the presence or absence of pAb589 peptide antigen (the C-terminal 22 amino acid residues of SR-BI) confirmed that the bands at apparent molecular weight of 140 and 210 kDa are SR-BI related protein which might be multimeric forms of SR-BI. ^{125}I apo A-I overlay analysis showed that SR-BI can bind to its ligand, apo A-I, only when it is thoroughly matured - glycosylated and dimerized. The antibody which was generated against extracellular domain of SR-BI (pAb230) not only prevented ^{125}I-labeled apo A-I from binding to 140 kDa band but also inhibited the esterified cholesterol uptake of rabbit BBMV with its IC50 value of 40 μg/ml of IgG. In contrast, the antibody generated against the C-terminal domain of SR-BI (pAb589) did not show any effect either on cholesterol uptake of rabbit BBMV or ^{125}I-labeled apo A-I binding to 140 kDa band. Overall results show that the ligand binding site of SR-BI in rabbit BBMV is located in extracellular domain, and SR-BI is only functional when it is part of dimeric forms which rationalize the previously found cooperative nature of the binding interaction and maybe a fundamental finding towards the so far poorly understood mechanism of SR-BI function.

Key words : scavenger receptor class B type I; brush border membrane; apolipoprotein A-I.

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Introduction

Intestinal sterol absorption by the brush border membrane (BBM) is an energy-independent, protein-mediated process based on various *in vitro* models such as brush border membrane vesicles (BBMV), intact enterocytes, and Caco-2 cells [2, 3, 4, 9, 11]. Our previous study identified a scavenger receptor of class B type I (SR-BI) as the integral membrane protein on the BBM of enterocytes responsible for the uptaking sterols and other hydrophobic lipids [6]. This receptor functions as a lipid port for a variety of classes of lipids including sterols, triacylglycerols and phospholipids [6]. Upon docking of the lipid donor particle, SR-BI mediates bidirectional flux of lipid molecules with little structural discrimination of the lipid molecules [6]. SR-BI has also been reported to be reverse cholesterol transport [7, 8].

The physiological ligand of SR-BI is high-density lipoprotein (HDL). In selective lipid uptake, HDL binds via apo A-I to SR-BI, and HDL-cholesteryl ester molecules are then transferred from the ligand to the acceptor membrane. The sterol uptake into small-intestinal BBMV is inhibited by free apolipoprotein A-I (apo A-I) or amphipathic α-helical peptides [1]. The minimal structural requirement of an inhibitor is an amphipathic α-helix of 18 amino acids, and the randomization of the amino acids sequence apparently abolishes the inhibition of sterol uptake [10]. The inhibition is competitive indicating that the inhibitors bind to SR-BI directly, and prevent the receptor from uptaking sterols [10]. Interestingly, the binding isotherm of apo A-I to SR-BI is sigmoidal which suggest that the binding is cooperative [10]. This cooperativity might be due to binding of the inhibitor molecule to a dimeric or oligomeric form of SR-BI (10). Here we address the question whether this complex is a functional unit of SR-BI in rabbit BBMV. Evidence is presented to show that SR-BI in rabbit BBMV is only functional when it is part of a complex which is linked by disulfide bridges. This result rationalizes the previously found cooperative nature of the binding interaction and maybe a fundamental finding towards the so far poorly understood mechanism of SR-BI function.

Materials and Methods

Preparation of brush border membrane vesicles (BBMV)

Rabbits were killed in slaughterhouse; the proximal small intestines of 1.5m length were excised and were rinsed thoroughly with 0.15 M NaCl, frozen in liquid nitrogen, and stored at -80℃ prior to the preparation of BBMV. The frozen small intestines (120-140g) were thawed and BBMV were prepared by the procedure of Hauser et al. [5].

In vitro translation

cDNA of SR-BI (CLA1) cloned in pZeoSV2(+) (Invitrogen) was digested with XhoI. After transcription of cDNA fragment using the T7 RNA polymerase, the resulting mRNA was isolated, and *in vitro* translation was carried out using rabbit reticulocyte lysate based on manufacturer's protocol (Promega) in the presence of ^{35}S-methionine. The lysates were separated on a 15% SDS-PAGE gel, and the gel was fixed with a solution containing 40% methanol/10% TCA for 30 min. After washing with 40% methanol/10% acetic acid, the gel was dried and was exposed onto the Phospho Imager (Molecular Dynamics, Inc.) to visualize the translated ^{35}S-labeled products.

SDS-PAGE and Western blot

Western blot was performed as described previously [10]. To see if the signal of pAb589 is chased away by its immunogen peptide, denatured rabbit BBMV was microcentrifuged, and supernatant was separated on 10 % SDS-PAGE and transferred onto a nitrocellulose membrane (Bio-Rad). After blocking with 2% BSA in Tris-buffered saline (50 mM Tris pH 7.4, 0.15 M NaCl) with 0.05% (v/v) Tween 20 (TTBS), membranes were incubated with pAb589 at a 1: 2,000 dilution in TTBS containing 20 μg/ml of immunogen peptide (CSKKGSKDKEAIQAYSESLMTA) for more than 3 hrs. After washing steps, the membranes were incubated for 40 min with an alkaline phosphatase-conjugated anti-rabbit IgG at a 1:10,000 dilution in TTBS containing 0.2% BSA. After additional washings with TTBS, the membranes were incubated with a chemiluminescent reagent according to the manufacturer's protocol (Bio-Rad), and were exposed to a Hyperfilm (Amersham).

Preparation of ^{125}I-labeled apo A-I and overlay analysis

^{125}I-labeled apo A-I was prepared as described previously [10]. Briefly, 200μg of human apo A-I in 100μℓ of PBS pH 7.4 with 500 μCi of ^{125}I-Na was incubated for 30 seconds in a IODO-GEN pre-coated iodination tube (Pierce) at room temperature. The reaction was stopped by transferring the mixture into a new IODO-GEN pre-coated iodination tube which includes 18.5 nmole of KI in 10 μℓ of PBS. The ^{125}I-labeled proteins were separated from free radioactivity by passing the reaction mixture through a PD-10 desalting column which was pretreated with 1% BSA in PBS and equilibrated with 50 ml of PBS. Fractions of 1 ml were collected, and the radioactivity of 10 μℓ aliquots were monitored using a gamma counter. The 4th and 5th fractions containing the highest radioactivities were combined (12 ± 2 % of recovery yield), and were used for apo A-I overlay analysis. For ^{125}I-labeled apo A-I overlay on Caco-2 cells, the cell lysates (50 μg of total protein/lane) were separated on 10 % SDS-PAGE and transferred onto a nitrocellulose membrane. The membrane was blocked with 2% of BSA and overlayed with 40 μg of ^{125}I-labeled apo A-I in 40 ml of TTBS for 3-5 hrs at room temperature. After several washing steps, the membrane was dried, and exposed either onto a film or onto a Phospho Imager (Molecular Dynamics) overnight. For ^{125}I labeled apo A-I overlay onto rabbit BBMV in the presence or absence of SR-BI antibodies, BBMV transferred membrane was preincubated in pAb230 or pAb589 (1 : 2,000 dilution) for 2 hrs before overlaying ^{125}I labeled apo A-I, and processed as described above. For the cold apo A-I binding assay, a blotted membrane was blocked with 2% of BSA, and was overlayed with 200 μg of cold apo A-I in 40 ml of TTBS for 3-5 hrs at room temperature. After 4 washes (10 min each), the membrane was incubated with anti apo A-I at a 1:1,000 dilution in TTBS for 1 h. After washing, the membrane was incubated for 40 min with alkaline phosphatase-conjugated anti-mouse IgG at a 1:10,000 dilution in TTBS and processed as described in the Western blotting protocol.

Inhibition of cholesterol ester uptake by various SR-BI antibodies

The inhibitory effect of various antibodies raised against different epitope of SR-BI on cholesterol ester uptake was determined as described previously [6, 10, 12]. Briefly, BBMV (5 mg of protein/ml) were incubated with egg PC small unilamellar vesicles (SUV) (50 μg/ml) containing 1 mol % radiolabeled sterol (esterified cholesterol), and the transfer of radiolabeled sterol to the BBMV was determined after 20 min in the presence and absence of increasing concentration of antibodies. The loss in esterified cholesterol uptake observed in the presence of each antibody was expressed as % of the total esterified cholesterol uptake observed in the absence of antibody (and equated with % inhibition). Dose response curves were constructed showing % inhibition as a function of the antibody concentration. For curve fitting the programs MacCurveFit (Kevin Raner Software, Victoria, Australia) and Excel (Microsoft) were used on a Macintosh computer as described in previously [1, 6].

Miscellaneous

Published methods were used for the preparations of small unilamellar vesicles (SUV) [3, 4, 9, 11], rabbit small-intestine BBMV [5, 9], and human apolipoprotein (apo) A-I [2]. Hydropathy plot was obtained using DNAstar software (DNAstar Inc.)

Results

In vitro translation and predicted topology of rabbit BBMV

In vitro translation study was performed to determine the topology of SR-BI. Based on the hydropathy plot of SR-BI, there are two hydrophobic regions which might be transmembrane regions in N- and C-terminus of the protein (Figure 1). In order to determine the orientation of SR-BI, cDNA of human SR-BI (CLA1) [6] was transcribed, and the resulting mRNA was translated *in vitro*. The full-length of SR-BI was successfully translated with its major band at 57 kDa (Figure 2A: lane 1). The translation product which was treated with Proteinase K showed an apparent molecular weight 4-5 kDa smaller than intact SR-BI (Figure 2A: lane 2) whereas no band was detectable from the sample which was treated both Triton X-100 and Proteinase K (Figure 2A: lane 3). The result and hydropathy profile suggests that the major portion of SR-BI is translocated into microsomes, and the C-terminus is located outside of microsomes (Figure 2C). The N-terminal part of SR-BI (size of 17 kDa) was translated, and the experiment was performed to determine the orientation of the N-terminal part of SR-BI. The sample which contains no microsomes shows an translation product at 17 kDa which is not glycosylated (Figure 2B: lane 1). The sample which contains microsomes produce not only the translation product at 17 kDa but also the products which are glycosylated (Figure 2B: lane 2). The results suggest that major part of the translated SR-BI is translocated into microsomes where the protein is glycosylated. When pretreated with acceptor peptide, however, the sample produce only 17 kDa product (unglycosylated form) and no glycosylated form was produced even in the presence of microsomes (Figure 2B: lane 3). After the carbonate extraction the pellet which is supposed to contain the microsomal membranes includes both glycosylated and unglycosylated proteins (Figure 2B: lane 4). However, the supernatant does not contain any shorter forms of a potential processed peptide (Figure 2B: lane 5) which suggests that no signal processing takes place. The results indicate that the N-terminal part of SR-BI is still in the membrane and a potential signal peptide is not cleaved. Both hydropathy plot and *in vitro* translation results show that the topology of SR-BI is predicted as shown in Figure 3. SR-BI is an integral membrane protein of 509 amino acids, consisting of a short cytoplasmic N-terminus of 9 amino acids followed by a first transmembrane domain of 22 amino acids, the extracellular domain of 408 amino acids, the second transmembrane domain of 22 amino acids, and the cytoplasmic C-terminus of 47 amino acids.

Western blot and apo A-I overlay analysis of rabbit BBMV

To confirm SR-BI in rabbit BBMV, we selected three anti SR-BI antibodies which were raised against different domain of SR-BI (Figure 3) based on predicted topology. As observed in our previous study [10], these antibodies preferentially detected different size of bands in immunoblot of rabbit BBMV. For instance, under conditions of short exposure times, antibody pAb589 detected reproducibly a 140 and 210 kDa bands (Figure 4A) whereas antibody pAbI15 detected preferentially the 84 and 100 kDa bands (Figure 4C) assigned to the monomeric form of SR-BI. The intensity of the 140 kDa band decreased at higher concentrations of DTT (Figure 4A). In contrast, the intensities of the 84 and 100 kDa bands increased reproducibly at higher concentrations of DTT (Figure 4C). These results suggest that the bands at apparent molecular weight of 140 or 210 kDa are probably a dimeric or tetrameric form of SR-BI linked by disulfide bridge(s) albeit the data presented cannot discriminate between a homomultimer and a heteromultimer of SR-BI. The apo A-I overlay analysis revealed that only the higher molecular weight bands (140 and 210 kDa) bind to apo A-I (Figure 4B). However, no binding of apo A-I to the monomeric form of SR-BI was detected in the overlay analysis indicating that apo A-I might bind only to multimeric form of SR-BI (Figure 4B). To confirm that the higher molecular weight bands (140 and 210 kDa) are SR-BI related bands, we performed the immunoblot of BBMV in the presence or absence of pAb589 peptide antigen (the C-terminal 22 amino acid residues of SR-BI) (Figure 3). The intensities of both bands were diminished by excess amount of peptide antigen (Figure 5). These results confirm that the band at an apparent molecular mass of 140 and 210 kDa bands are SR-BI related protein which might be multimeric forms of SR-BI.

^{125}I apo A-I overlay onto Caco-2 cells

Western blot was done using pAb230 to observe the maturation of SR-BI based on the different cell differentiation status (Figure 6A). Undifferentiated Caco-2 cells showed band only at 57 kDa (Figure 6A; lanes 1, 2), whereas differentiated cells showed several different sizes (>57 kDa) of bands (Figure 6A; lanes 3, 4). The increasing sizes of SR-BI derivatives might be resulted from the maturation of protein (e.g. glycosylation and dimerization). ^{125}I apo A-I overlay analysis was performed to observe the ligand binding to SR-BI based on the different cell differentiation status (Figure 6B). The result showed that apo A-I binds only to the protein of apparent molecular weight of 140 kDa in differentiated cells which was treated with 1 mM DTT (Figure 6B; lane 3). In contrast, the intensity of signal was diminished when the sample was treated with 100 mM DTT (Figure 6B; lane 4) as observed in Figure 4B. The results show that SR-BI can bind to its ligand, apo A-I, only when it is thoroughly matured - glycosylated and dimerized.

Inhibition of ^{125}I apo A-I binding and sterol absorption by pAb230

Also we compared ^{125}I apo A-I overlay onto BBMV in the

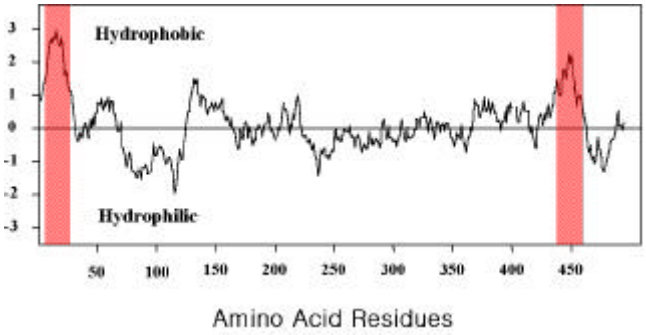
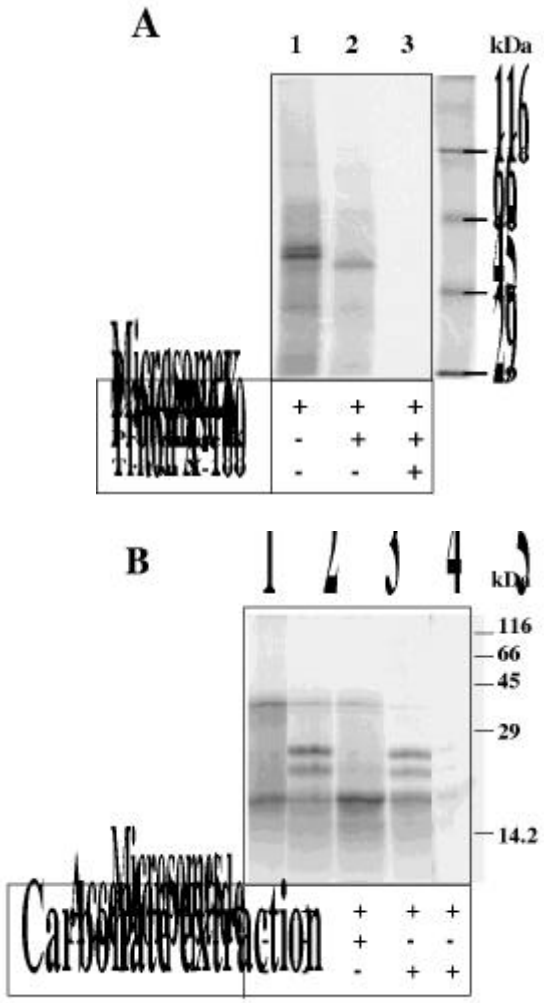


Fig. 1. Hydrophathy of SR-BI and predictions of its membrane spanning regions. Kyte-Doolittle hydrophathy plot is shown as a function of amino acid residues. The predicted membrane spanning regions are indicated by the shaded bars.



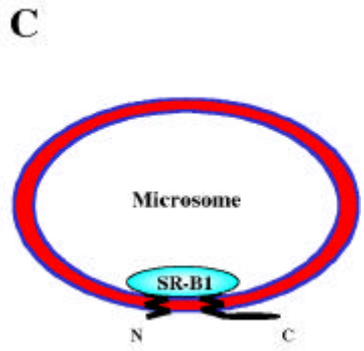


Fig. 2. *In vitro* translation of SR-BI mRNA. cDNA of SR-BI (CLA1) cloned in pZeoSV2(+) (Invitrogen) was digested with XhoI. After transcription of cDNA fragment using the T7 RNA polymerase, the resulting mRNA was isolated, and *in vitro* translation was carried out using rabbit reticulocyte lysate based on manufacturer's protocol (Promega) in the presence of 35S-methionine. The lysates were separated on a 15% SDS-PAGE gel, and the gel was fixed with a solution containing 40% methanol/10% TCA for 30 min. After washing with 40% methanol/10% acetic acid, the gel was dried and was exposed onto the Phospho Imager (Molecular Dynamics, Inc.) to visualize the translated 35S-labeled products. Panel A: *in vitro* translation of full-length of SR-BI. Panel B: *in vitro* translation of the N-terminal domain of SR-BI (size of 17 kDa). Panel C: predicted topology of translated SR-BI in microsome, the major portion of SR-BI is translocated into microsomes, and the C-terminus is located outside of microsomes.

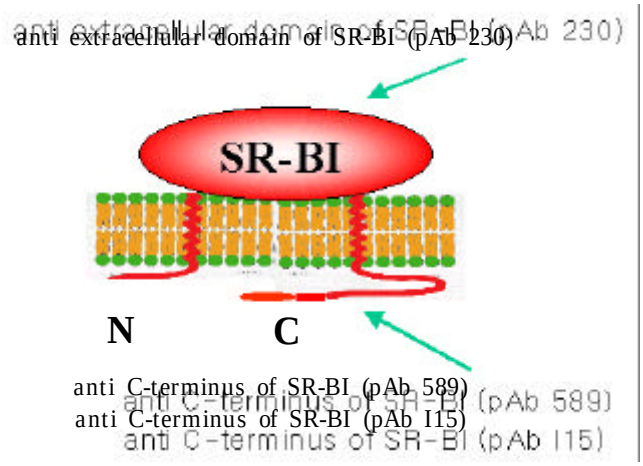


Fig. 3. Predicted topology of SR-BI and antibodies raised against different domains of SR-BI. The receptor is an integral membrane protein of 509 amino acids, consisting of a short cytoplasmic N-terminus of 9 amino acids followed by a first transmembrane domain of 22 amino acids, the extracellular domain of 408 amino acids, the second transmembrane domain of 22 amino acids, and the cytoplasmic C-terminus of 47 amino acids.

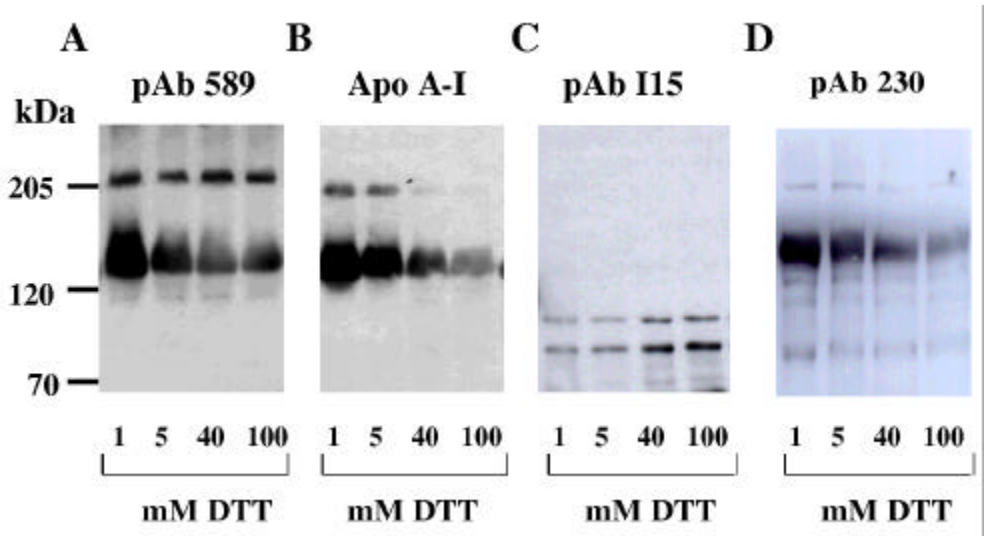


Fig. 4. Immunoblot and apo A-I overlay analysis of BBMV. Rabbit BBMV were treated with 1, 5, 40, or 100 mM of DTT in SDS sample buffer, and were subjected to Western blotting using either anti SR-BI antibody pAb 589 which specifically detects a 140 and 210 kDa band (panel A), or antibody pAb I15 which primarily detects a 82 and 100 kDa band (panel B). Panel C shows the apo A-I overlay analysis of BBMV. The strip was overlayed with cold apo A-I, and bound apo A-I was detected using anti apo A-I antibody as described in "Materials and Methods". Equal amounts (50 μg/lane) of protein were applied in each lane. Panels A to C are representative of three reproducible experiments in which two different batches of BBMV were used.

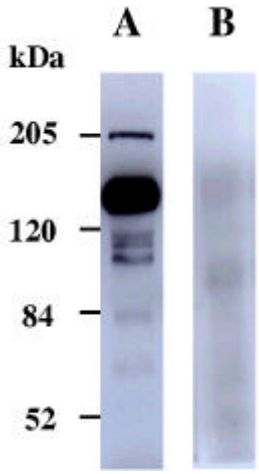


Fig. 5. Immunoblot of BBMV in the presence or absence of pAb589 peptide antigen. Each strip of blot was incubated with pAb589 (1: 2,000 dilution) in the absence (panel A) or presence (panel B) of 20 μg/ml of peptide antigen, and was visualized using secondary antibody as described in "Materials and Methods".

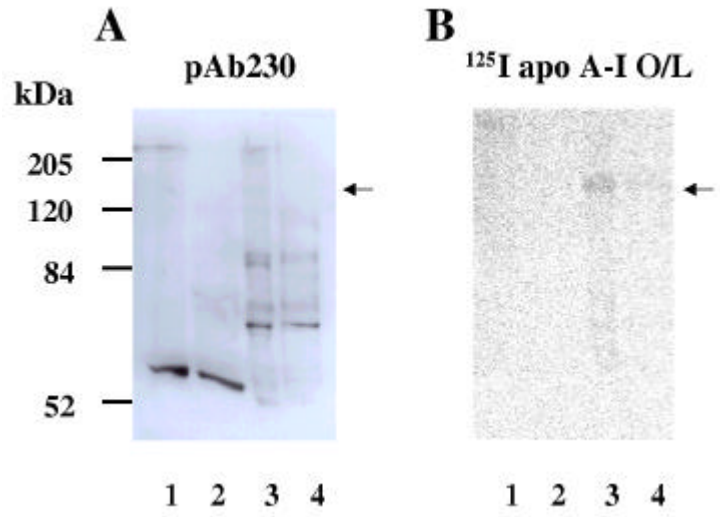


Fig. 6. Immunoblot and 125I-labeled apo A-I overlay on Caco-2 cell. Panel A, undifferentiated (lanes 1 and 2) and differentiated (lanes 3 and 4) Caco-2 cells were treated with SDS sample buffer containing 1 mM of DTT (lanes 1 and 3) or 100 mM of DTT (lanes 2 and 4), and were immunoblotted using pAb230 as described in "Materials and Methods". Panel B, the blot was overlayed with 125I-labeled apo A-I, and the signal was detected using the Phospho Imager (Molecular Dynamics) as described in "Materials and Methods".

absence or presence of SR-BI antibody to confirm that the band at 140 kDa which binds to apo A-I is SR-BI. In the presence of pAb230, which was generated against extracellular domain of SR-BI, the signal of 125I apo A-I from 140 kDa was competed away (Figure 7B). In contrast, pAb589, which is generated against the C-terminal sequence of SR-BI, could not chase away the signal (Figure 7C). The results show that the apo A-I binding site of SR-BI resides in extracellular part of protein (residues 230-380) rather than C-terminus of SR-BI. To test the effect of blocking in different domain of SR-BI on cholesterol uptake, we observed the esterified cholesterol uptake of rabbit BBMV in the presence of three different antibodies. The esterified cholesterol uptake of BBMV was inhibited by pAb230 with its IC50 value of 40 μg/ml of IgG, whereas the uptake was not inhibited either by pAbI15 or by pAb589 which was generated against the C-terminal domain of SR-BI (Figure 8).

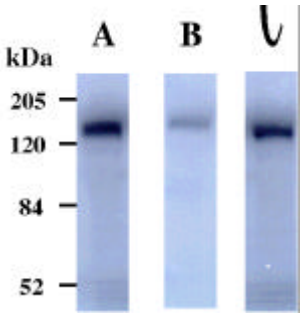


Fig. 7. 125I-labeled apo A-I overlay onto BBMV in the absence or presence of antibody against extracellular domain of SR-BI. Each blot was overlayed with 40 μg of 125I-labeled apo A-I in 40 ml of TTBS containing 0.2% BSA for 3-5 hrs at room temperature in the absence (Panel A) or presence (Panel B) of pAb230 (200 μg/ml, 1: 2,000 dilution) or in the presence of pA589 (Panel C). After several washing steps, the membrane was dried in a gel dryer for 30 min at 60℃, and was exposed onto a film overnight.

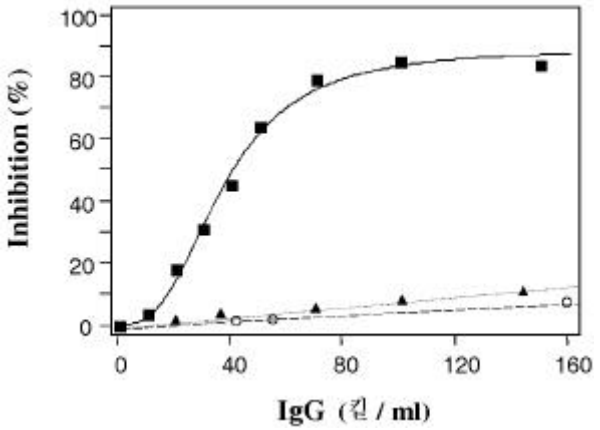


Fig. 8. Inhibition of esterified cholesterol uptake by BBMV as a function of concentration of antibody against various domain of SR-BI. Dose-response curves were constructed from rates of esterified cholesterol uptake in the presence of pAb230 (filled squares), pAb589 (open circles), and pAbI15 (filled triangles). Rates of esterified cholesterol were calculated from data points (average ± SD, n=3) obtained after incubation for 20 min. The curve fitting was done using a modified Hill equation as described previously [6]. The error bars were smaller than the size of the symbols and therefore omitted.

Overall results show that the ligand binding site of SR-BI in rabbit BBMV is located in extracellular domain, and SR-BI is only functional when it is part of dimeric forms which rationalize the previously found cooperative nature of the binding interaction and maybe a fundamental finding towards the so far poorly understood mechanism of SR-BI function.

Discussion

Present study predicted the topology of SR-BI; large extracellular domain is anchored to plasma membrane at both N- and C-terminal ends which have short extensions into the cytoplasm N- and C-terminal residues. Overall results show that the ligand binding site of SR-BI in rabbit BBMV is located in extracellular domain, and SR-BI is only functional when it is part of dimeric forms which rationalize the previously found cooperative nature of the binding interaction.

Our previous study [10] showed that binding of apo A-I to SR-BI of rabbit BBMV is cooperative, characterized by a dissociation constant *Kd* = 0.45 M and a Hill coefficient of n = 2.8. After proteinase K treatment of BBMV, the affinity of the interaction of apo A-I expressed as *Kd* is reduced by a factor of 20, and the cooperativity is lost [10]. The Western blot and apo A-I overlay analysis shed light on the origin of the cooperativity of apo A-I binding to SR-BI. The cooperativity could be due to several ligand binding sites per SR-BI molecule or alternatively to apo A-I binding to SR-BI oligomers. The results of the present study are consistent with the latter case supporting the notion that SR-BI might be functional in its dimeric or oligomeric form. This is strongly supported by previous study which observed that cross-linking of mouse SR-BI with apo A-I makes apparent molecular weight of 225 kDa protein complex [13]. Assuming that 60 kDa of this complex is due to apo A-I cross-linked to itself, approximately 165 kDa might be due to mouse SR-BI dimer [13]. Since a monomer of mouse SR-BI (glycosylated one) has an apparent molecular weight of 82 kDa, 165 kDa is sufficient mass to reflect the dimeric form of mouse SR-BI or a monomeric form of SR-BI complexed with one or more other membrane proteins.

Here, a question can be arise; why pAb589 which was generated against the C-terminal domain (from 477 to 495) of SR-BI only detects the bands at 140 and 210 kDa whereas pAbI15 (generated against the domain from 496 to 509) only detects the bands at 84 and 100 kDa ? Our model

explain that there is an equilibrium between the portion of monomeric form and oligomeric form under the condition of SDS sample buffer treatment and SDS-PAGE separations. If there is a conformational difference between monomer and oligomer (e.g. if C-terminal domain (from 477 to 495) is shield inside in its monomeric form and is exposed outside in its oligomeric form), pAb589 can detect only oligomeric form of SR-BI. In case of Western blot using pAbI15, the phenomenone is opposite to the case of pAb589. The present study shows that the signal of pAb589 is chased away by excess amount of peptide antigen (Figure 5) which support that the bands at an apparent molecular weight of 140 and 210 kDa bands are SR-BI related ones. Therefore, a possible explanation is that two adjacent epitopes are folded differently based on monomeric or oligomeric form of SR-BI. In contrast, pAb230 which was generated against 150 amino acids residues (from 230 to 380) of extracellular domain can detect the bands at 140, 210, and 84 kDa. The result indicates that the extracellular domain which is hydrophilic is exposed outside – that is enough to be detected by pAb230 regardless of its monomer or oligomer.

Based on our observations, apo A-I binds only to the extracellular domain of SR-BI (somewhere between 230 to 380) since only pAb230 can inhibit lipid uptake by BBMV whereas other antibodies which were generated against C-terminal of SR-BI can not block the lipid uptake. Moreover, apo A-I can bind only dimerized form of SR-BI since pAb230 can chase away the signal of ^{125}I apo A-I to at 140 kDa band whereas pAb589 does not. Another evidence was derived from apo A-I overlay analysis onto Caco-2 cells. Apo A-I can bind only to 140 kDa band which was derived from differentiated cells in the presence of low concentration of DTT. The result shows that apo A-I can bind to matured SR-BI which is glycosylated and dimerized. Therefore, the dimerized form of SR-BI must be the functional unit which can interact with its ligand.

Overall, apo A-I only binds to the extracellular domain of dimerized form of SR-BI. However, it is still unclear why apo A-I can interact only with the dimerized form of SR-BI. Maybe there need a cooperativity for the interaction between SR-BI and apo A-I. In our previous study [10], proteinase K treatment of BBMV not only abolishes the cooperativity of the apo A-I binding but also reduces *Kd* value by a factor of 20. In their sigmoidal binding isotherm, first binding of apo A-I has low *Kd* value whereas second binding has higher *Kd* value to SR-BI. Therefore, losing cooperativity must have lower *Kd* value (low *Kd* value for the first apo A-I binding or even lower) for the interaction between apo A-I and SR-BI, and multimeric form of SR-BI should have higher Kd value than monomeric form of SR-BI.

Also there might be a conformational difference between monomeric and multimeric form of SR-BI. There are 41 cysteine in human SR-BI; 44 cys in mouse SR-BI; unknown in rabbit SR-BI. There must be a lot of inter- or intra-disulfide bridges which might be important for maintaining the three dimensional structure of SR-BI for binding its ligands since higher concentration of DTT treatment of BBMV resulted in poorer binding of apo A-I. Therefore, our conclusion is that 140 kDa band is a functional unit, and a dimeric form of SR-BI which is composed of two monomers linked with disulfide bridge(s). But we still don't know whether it is a homo- or heterodimer. Additional studies to elucidate the nature of this protein complex are clearly warranted.

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