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Expression, purification and reconstitution of the C-terminal transmembrane domain of scavenger receptor BI into detergent micelles for NMR analysis

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ABSTRACT

Scavenger receptor class B type I (SR-BI), the high density lipoprotein (HDL) receptor, is important for the delivery of HDL–cholesteryl esters to the liver for excretion via bile formation. The focus on therapeutic strategies aimed at reducing cholesterol levels highlights the critical need to understand the structural features of SR-BI that drive cholesterol removal. Yet, in the absence of a high-resolution structure of SR-BI, our understanding of how SR-BI interacts with HDL is limited. In this study, we have optimized the NMR solution conditions for the structural analysis of the C-terminal transmembrane domain of SR-BI that harbors putative domains required for receptor oligomerization. An isotopically-labeled SR-BI peptide encompassing residues 405–475 was bacterially-expressed and purified. [*U*-^{15}N]-SR-BI(405–475) was incorporated into different detergent micelles and assessed by ^{1}H–^{15}N-HSQC in order to determine which detergent micelle best maintained SR-BI(405–475) in a folded, native conformation for subsequent NMR analyses. We also determined the optimal detergent concentration used in micelles, as well as temperature, solution buffer and pH conditions. Based on ^{1}H–^{15}N-HSQC peak dispersion, intensity, and uniformity, we determined that [*U*-^{15}N]-SR-BI(405–475) should be incorporated into 5% detergent micelles consisting of 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phospho-[1′-rac-glycerol] (LPPG) and data collected at 40 °C in a non-buffered solution at pH 6.8. Furthermore, we demonstrate the ability of SR-BI(405–475) to form dimers upon chemical crosslinking. These studies represent the first steps in obtaining high-resolution structural information by NMR for the HDL receptor that plays a critical role in regulating whole body cholesterol removal.

Introduction

Decades of epidemiological data strongly suggest that high density lipoproteins (HDL)[1] protect against cardiovascular disease (reviewed in [1]). While HDL exerts numerous beneficial effects via inhibition of oxidative, apoptotic and inflammatory pathways (reviewed in [1]), its primary cardio-protective effect is attributed to its role in reverse cholesterol transport [2]. In this pathway, HDL removes cholesterol from peripheral tissues and transports it to the liver for excretion via bile formation [2]. Scavenger receptor class B type I (SR-BI), the HDL receptor, facilitates this transfer of cholesterol from HDL into cells. The physiological importance of SR-BI in mediating cholesterol flux in humans was obtained from the recent identification of three loss-of-function mutations in *SCARB1* (the human SR-BI gene) in heterozygote carriers with high HDL-cholesterol (HDL-C) levels [3,4]. These mutant SR-BI receptors were unable to mediate selective uptake of HDL–cholesteryl esters (CE) in cultured cells [4,5]. While the risk for cardiovascular disease has not been assessed in this small cohort of patients, the anti-atherogenic properties of SR-BI and its ability to promote reverse cholesterol transport are firmly established in genetically-modified

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[1] *Abbreviations used:* ANS, 1-anilinonaphthalene-8-sulfonic acid; CD, circular dichroism; CE, cholesteryl ester; CMC, critical micelle concentration; DDM, n-dodecyl β-D-maltopyranoside; DPC, n-dodecylphosphocholine; HDL, high density lipoprotein; HDL-C, HDL-cholesterol; HSQC, heteronuclear single quantum coherence; IPTG, isopropyl β-D-1-thiogalactopyranoside; LMPG, 1-myristoyl-2-hydroxy-*sn*-glycero-3-phospho-(1′-rac-glycerol); LOPG, 1-oleoyl-2-hydroxy-*sn*-glycero-3-phospho-(1′-rac-glycerol); LPPG, 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phospho-(1′-rac-glycerol); NMR, nuclear magnetic resonance; OG, octyl β-D-glucopyranoside; SR-BI, scavenger receptor class B type I.

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mouse models. Hepatic overexpression of SR-BI in mice [6–8] markedly lowered HDL-C, increased cholesterol catabolism and excretion, and reduced atherosclerosis [9–11]. On the other hand, a 50% reduction in SR-BI expression [12] or full disruption of the SR-BI gene [13,14] in mice significantly increased plasma HDL-C levels, yet dramatically accelerated atherosclerosis [14–16]. Since the delivery of HDL–CE to hepatic tissues only occurs upon binding of HDL to SR-BI, understanding the HDL/SR-BI interaction is critical as we strive to develop novel strategies to reduce whole body cholesterol levels.

There is currently no high-resolution structure available for SR-BI, an 82-kDa cell surface glycoprotein [17]. Based on hydropathy analyses [18], SR-BI (509 amino acids) consists of a short N-terminal cytoplasmic tail (~8 residues), followed by an N-terminal transmembrane domain (~28 residues), a large extracellular domain (~403 residues), a C-terminal transmembrane domain (~25 residues) and finally, a C-terminal cytoplasmic tail (~45 residues) (Fig. 1A) [19]. Binding of HDL to the extracellular domain of SR-BI is necessary [20–24], but not sufficient for selective uptake of HDL–CE. It has been speculated that formation of a productive HDL/SR-BI complex [25] depends on the correct alignment of specific lipoprotein and receptor domains and/or the capacity of the receptor to undergo appropriate conformational changes that permit efficient lipid transport. Several studies [26–29], including our own [5,30–32], have demonstrated the important contributions of specific extracellular regions of SR-BI in mediating the selective uptake of HDL–CE. Indeed, the recent availability of the X-ray crystal structure of the extracellular domain of LIMP-2 [33], a scavenger receptor that shares 30% sequence identity with SR-BI, provides the opportunity to better recognize key structural features of this domain that contribute to its cholesterol transport functions.

The efficiency of HDL–CE selective uptake is also dependent on SR-BI oligomerization [34]. We [35,36] and others [37–39] have demonstrated the presence of SR-BI oligomers, and it has been postulated that HDL–CE uptake occurs via a non-aqueous pathway, possibly involving the formation of a "hydrophobic channel" [40]. Importantly, live cell fluorescence resonance energy transfer studies indicate that self-association of SR-BI is mediated by interaction between the C-terminal transmembrane domains [35], while another study identified a glycine dimerization motif in the N-terminal transmembrane domain that mediates SR-BI oligomerization [41]. In order to understand the mechanisms that regulate SR-BI oligomerization and the selective uptake of HDL–CE, it is critical that we understand the structure of the transmembrane domains of SR-BI.

In this study, we report the expression and purification of SR-BI(405–475), a peptide that encompasses residues 405–475 of SR-BI that contains the C-terminal transmembrane domain of SR-BI (residues 441–465), as well as portions of the extracellular domain (residues 405–440) and the C-terminal cytoplasmic domain (residues 466–475). We also describe optimization of conditions that will enable us to obtain high-resolution structural information of this peptide using nuclear magnetic resonance (NMR)-based strategies. These studies represent the first steps in obtaining structural information for the HDL receptor that plays a critical role in regulating whole body cholesterol removal.

Methods

Materials

The following detergents were used: 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phospho-(1′-rac-glycerol) (LPPG; Avanti Polar Lipids, Inc.); octyl β-D-glucopyranoside (OG; Sigma–Aldrich); n-dodecylphosphocholine (DPC; Affymetrix); empigen (Sigma–Aldrich); n-dodecyl β-D-maltopyranoside (DDM; Sigma–Aldrich); 1-myristoyl-2-hydroxy-*sn*-glycero-3-phospho-(1′-rac-glycerol) (LMPG; Affymetrix); 1-oleoyl-2-hydroxy-*sn*-glycero-3-phospho-(1′-rac-glycerol) (LOPG; Avanti Polar Lipids, Inc.). His60 Ni Superflow resin was purchased from Clontech. Protease inhibitor cocktail was purchased from Fermentas and RNAse was from Thermo Scientific. DNAse, glutaraldehyde, and 1-anilinonaphthalene-8-sulfonic acid (ANS) were purchased from Sigma–Aldrich. All other reagents were of analytical grade.

Plasmids

Sequences encompassing the C-terminal transmembrane domain of murine SR-BI cDNA were PCR-amplified and cloned into the *Bam*HI and *Hind*III sites of a modified pQE30 vector (QIAGEN). The resulting plasmids encoded for peptides with an N-terminal eight-residue histidine tag and tobacco etch virus cleavage site, followed by SR-BI sequences of varying length. Protein purification, circular dichroism, and NMR experiments were performed using a peptide corresponding to SR-BI residues 405–475 [herein

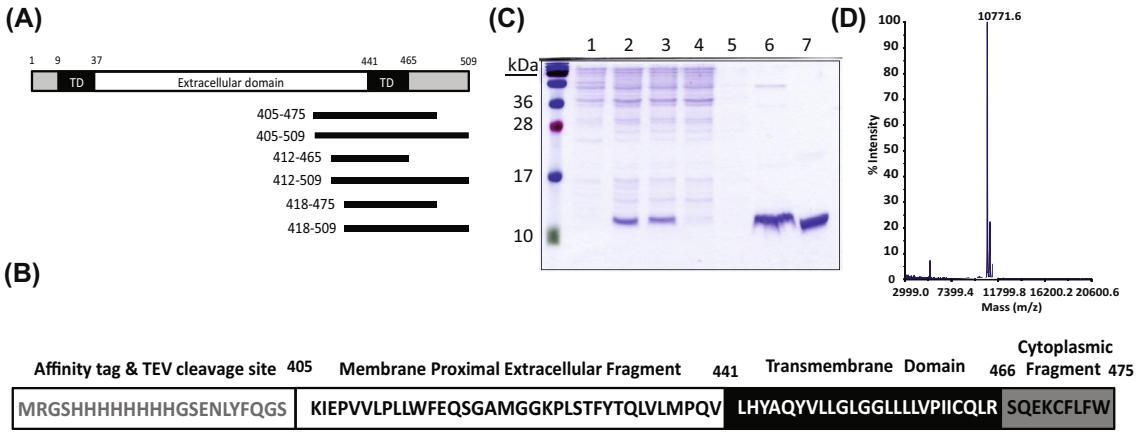

Fig. 1. Purification of SR-BI(405–475). (A) Schematic representation of linear murine SR-BI, where cytoplasmic domains (grey), transmembrane domains (TD; black) and the extracellular domain (white) are shown. SR-BI peptides containing the C-terminal transmembrane domain were cloned into a modified pQE30 vector and ranged from 53 to 104 residues in length. (B) The sequence of SR-BI(405–475), which includes the affinity tag and TEV cleavage site, membrane proximal extracellular fragment, transmembrane domain, and cytoplasmic fragment. (C) SR-BI(405–475) was purified as described in Materials and Methods. Lane 1, whole cell lysate of transformed SR-BI(405–475) in *E. coli*; lane 2, whole cell lysate following protein expression induced by IPTG; lane 3, supernatant fraction following solubilization of cell pellet in empigen detergent; lane 4, Ni-column flow through; lane 5, Ni-column wash; lane 6, Ni-column elution of protein with 500 mM imidazole; lane 7, HPLC-purified peptide. (D) MALDI mass spectrometry analysis of SR-BI(405–475) peptide (expected mass is 10,778 Da).