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Cloning, Expression and Purification of the SRCR domains of glycoprotein 340

Sangeetha Purushotham^a and Champion Deivanayagam^{a,*}

^aDepartment of Vision Sciences and Center for Structural Biology 1025 18th Street South, CBSE/UAB University of Alabama at Birmingham Birmingham, AL 35294-4400

Abstract

Glycoprotein 340 (gp340), an innate immunity molecule is secreted lumenally by monolayered epithelia and associated glands within the human oral cavity. Gp340 contains 14 scavenger receptor cysteine rich (SRCR) domains, two CUB (C1r/C1s Uegf Bmp1) domains and one zona-pellicular (ZP) domain. Oral streptococci are known to adhere to the tooth immobilized gp340 via its surface protein Antigen I/II (AgI/II), which is considered to be the critical first step in pathogenesis that eventually results in colonization and infection. In order to decipher the interactions between gp340's domains and oral streptococcal AgI/II domains, we undertook to express human gp340's first SRCR domain (SRCR₁) and the first three tandem SRCR domains (SRCR₁₂₃) in *Drosophila* S2 cells. While our initial attempts with human codons did not produce optimal results, codon-optimization for expression in *Drosophila* S2 cells and usage of inducible/secretory *Drosophila* Expression System (DES) pMT/BiP/V5-HisA vector greatly enhanced the expression of the SRCR domains. Here we report the successful cloning, expression, and purification of the SRCR domains of gp340. Recognition of expressed SRCRs by the conformational dependent gp340 antibody indicate that these domains are appropriately folded and furthermore, surface plasmon resonance studies confirmed functional adherence of the SRCR domains to AgI/II.

Introduction

The human oral cavity contains a number of organisms that colonize the tooth surface, which results in complex interspecies interactions and results in the formation of dental plaques on tooth enamel [1, 2]. Oral streptococci, considered to be the early colonizers initiate attachment to tooth surface immobilized salivary agglutinin (SAG, gp340) through the surface protein Antigen I/II (AgI/II) [3, 4]. Our lab has been interested in structurally and functionally characterizing the interaction between AgI/II of the caries pathogen *Streptococcus mutans* (*S. mutans*) and gp340. In this regard, we have recently determined the structures of AgI/II's adherence domains [5, 6]. To further elucidate the mechanistic details of this interaction, we have now embarked on structurally and functionally characterizing the human receptor gp340 and its subdomains.

Gp340 is a ~360 KiloDalton (kDa) glycoprotein that is secreted lumenally by monolayered epithelia and associated glands and has 14 Scavenger receptor cysteine rich (SRCR)

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*Corresponding Author champy@uab.edu Phone:205-934-6026 Fax: 205-934-0480.

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domains, two CUB (C1r/C1s Uegf Bmp1) domains and one Zona pellucida (ZP) domain (Figure 1) [7]. The CUB domain contains approximately 100-110 amino acids with four conserved disulfide bonds. These domains were named so, as they were first observed in the complement pathway subcomponent (C1s/C1r), in sea urchin epidermal growth factor (Uegf) and in bone morphogenetic protein (Bmp1) [8]. The ZP domain contains approximately 260 amino acid residues with eight conserved cysteines and are usually present at the C-terminus of glycosylated proteins, and is attributed to play a role in protein oligomerizations [8, 9]. Among the SRCR domains within gp340, there exists high homology and the SRCR's are in general interspersed with domains termed as SIDs [10]. The glycosylations that decorate gp340 are considered to contribute approximately 20-40% of its molecular weight. Gp340's SRCR domains are predicted to contain N-glycosylation sites within SRCR domains and O-linked glycosylations primarily within the SIDs [10-12]. These SRCR domains (~100-110 amino acids) belong to an ancient fold, and are classified according to the number of cysteines, where gp340's SRCR domains belong to the group B (8 cysteines) and are different from group A (6 cysteines) [11, 13]. The SRCR domains are present in various allelic forms from humans down to invertebrates and exist both in membrane-bound and secreted forms [8, 14].

With the observation of aggregation of various types of bacteria including cariogenic, viridians group streptococci and viruses [12, 15], gp340 is now acknowledged to be innate immunity factor within the oral cavity [16]. Recently, gp340 was shown to aid transcytosis of HIV across vaginal epithelial cells [17]. Apart from the oral cavity, gp340 is also found in lungs [11], tears [18], vagina [19, 20] and brain (known as DMBT1) [7]. While its functionality in these different regions is still being investigated, our focus is to identify the mechanistic details of its interaction with the caries pathogen *S. mutans*. In this paper, we report the expression and purification of SRCR domains utilizing the *Drosophila* S2 cells expression system and present evidence of their functionality.

Material and Methods

DMBT1 template vector

The pTR8kb.2_3ssTO, a tetracycline-inducible expression pT-REx-DEST-30 vector harboring the *DMBT1/gp340* gene (a gift from Dr. Poustka's lab [21]) was used as template for cloning the SRCR domains.

SRCR constructs

To assess the adherence properties of single as well as multiple SRCR domains, we chose to express two constructs SRCR₁ and SRCR₁₂₃, which encompassed residues 95-226 and 95-486 respectively of gp340 (Figure 1).

Initial Cloning of SRCR domains into pMT/V5-HisA vector

The template vector pTR8kb.2_3ssTO (1 µg/ml) was used for PCR with cycling conditions set at 95 degree Celsius (°C) for 2 minutes, denaturing at 95 °C for 30 seconds and primer annealing at 55 °C for 30 seconds with extension at 72 °C for 3 minutes and final extension for 10 minutes at 72 °C over 30 cycles. PCR reactions were done using 0.5 µl of Dynazyme polymerase (New England Biolabs, Inc) and 100 mM MgSO₄. PCR products obtained from these reactions run over a DNA agarose gel indicated the presence of ~339 base pair (bp) and ~1125 bp bands, which corresponded to the SRCR₁ and SRCR₁₂₃ domains respectively. Using restriction enzymes *KpnI* and *AgeI*, the PCR products and the pMT/V5-HisA vector (Invitrogen), which is an inducible *Drosophila* expression system (DES) vector containing a C-terminal 6 ×His-tag and an incorporated ampicillin selection gene were digested and thereafter ligated at 1:1 molar ratio using T4 DNA ligase (New England Biolabs, Inc). The

ligated products were purified and transformed into chemically competent *E. coli* DH5 α cells (Invitrogen, Inc). Appropriate insertion of *SRCR* genes into the pMT/V5-HisA vector were verified by DNA sequencing (UAB Heflin sequencing center). The plasmid DNA was extracted from these transformed *E. coli* DH5 α cells.

S2 cell culture

S2 cell lines derived from the late stage (20-24 hours) primary cultures of *Drosophila melanogaster* embryos [22] are semi-adherent monolayer cells and grow as suspensions in carbon-dioxide and humidity free environment at temperatures close to 23 °C. These cells were grown in a complete growth media (Invitrogen, Inc), which contains Schneider's *Drosophila* medium with 10% heat-inactivated fetal bovine serum (FBS).

Generation of stable S2 cells expressing SRCR domains

The recombinant expression vectors pMT/V5-HisA containing SRCR₁ or SRCR₁₂₃ were each transfected stably into S2 cells. Briefly, the S2 cells (3×10^6 cells/ml) were grown in a 6-well culture plate containing 3 ml of complete media (Invitrogen, Inc) until the cell density reached 5×10^6 cells/ml. The pCoBlast plasmid, a highly efficient and faster co-transfection vector that expresses blasticidin-S-deaminase in S2 cells under the control of copia promoter for stably selecting recombinant DNA transfected cells was used for stable selection[23, 24]. Calcium Phosphate Transfection kit (Invitrogen, Inc) was used to transfect the S2 cells stably as per manufacturer's protocol. The cells were transferred into a selection medium (10 μ g/ml blasticidin in complete media), wherein blasticidin resistant clones appeared in about 2 weeks. The clones were further expanded in selection medium and copper sulfate (500 μ M) was used to induce the expression of SRCR domains on the stably transfected S2 cells (2×10^6 cells/ml). These cultures were finally harvested after 7 days. The cell lysate and growth media samples were obtained as described in sample preparation.

Sample preparation

Copper sulfate induced stably transfected S2 cells were centrifuged at 800 rpm for 3 minutes, and the supernatant containing growth media was collected. This growth media was then thoroughly dialyzed multiple times using an 8,000 Da molecular weight cut off (MWCO), membrane tubing (Spectrum, Inc) into a buffer containing 20 mM Tris pH 8.0, 500 mM NaCl and 1% glycerol to completely remove the copper ions. Separately, cell lysates were prepared by washing (2 $\times$) the pelleted cells in Phosphate buffered saline (PBS) and thereafter incubating in 1 ml of cell lysis buffer containing 50 mM Tris pH 7.4, 150 mM NaCl, 1% Nonidet P-40 and protease inhibitors (1 mM PMSF, 1 μ g/ml pepstatin and 1 μ g/ml of leupeptin) for 10 minutes on ice. These lysed cells were centrifuged at 12,000 rpm for 10 minutes at 4 °C and their supernatant was collected for further analysis.

SDS-PAGE

SRCR₁ and SRCR₁₂₃ (~2 μ g/ml) were dissolved in reducing sample solubilizing buffer containing 0.125 M Tris-HCl pH 6.8, 10% glycerol, 2% β -mercaptoethanol, 2% SDS, 0.1% bromophenol blue and were heat treated at 95 °C for 5 minutes prior to electrophoresis. These samples were then electrophoretically separated on a 12.5% SDS-PAGE gel at 100 V for 65 minutes on a mini-gel (Biorad, Inc), and stained with 0.1% Coomassie brilliant blue dye (Sigma) for visualization.

Isolation of RNA and RT-PCR

Stably transfected S2 cells harboring the recombinant vector were grown to 80% confluency in a 6 well culture plate. The total RNA was isolated using TRIzol reagent (Invitrogen, Inc) as per manufacturer's protocol. 1 μ g/ μ l of the total RNA was used as template for the

synthesis of first-strand of cDNA with the master mix and random hexamer (GeneAmp RNA PCR kit) and cycling conditions set at 44 °C for 60 minutes and 92 °C for 10 minutes. Following which PCR amplification of the *SRCR* genes were performed with gene specific forward primer common for both *SRCR*₁ and *SRCR*₁₂₃ (5'-CGCGGTACCGTCATGGGATCTGATTCTGGTTTG-3') and respective reverse primers for *SRCR*₁ (5'-CGAACCGGTCTGAGGCTGGGCAGCTGA-3') and *SRCR*₁₂₃ (5'-GAACCGGTCGACCAGGAGTGGGCAGC-3'). The resultant product was finally analyzed using DNA agarose gel electrophoresis.

Cloning of codon optimized *SRCR* domains into pMT/BiP/V5-HisA and establishment of stable *Drosophila* S2 cells

SRCR genes were synthesized, wherein these codons were optimized using Gene Optimizer expert software from GENEART that employed sliding window algorithm [25]. This algorithm covers several amino acid positions slinging along the coding sequence forming a "variation window". The sequence-optimized gene (See supplemental information) was then synthesized, assembled by primer extension-based PCR and cloned in the pMT/BiP/V5-HisA vector. The pMT/BiP/V5-HisA contains the BiP signal sequence (MKLCILLAVVAFVGLSLG) that is designed for use with *Drosophila* Inducible/Secreted Expression System (DES®), where the protein of interest is secreted into the growth media on induction with copper sulfate (500 µM). Stable cell lines harboring the codon-optimized genes for *SRCR*₁ and *SRCR*₁₂₃ were established as previously described.

Large scale expression and purification of *SRCR*s

Large scale expression of *SRCR*₁ and *SRCR*₁₂₃ were carried out by expanding the stably transfected S2 cells in large suspension cultures. Initially, the cells were grown to 80% confluency in selection medium in T-75 cell culture flasks, and then 5×10^6 cells/ml were inoculated into a spinner flask containing Insect XPRESS protein-free medium with L-glutamine (Lonza), where the surfactant (0.1% Pluronic F-68) was added to prevent cell shearing. The spinner flask was then incubated at 22 °C at constant stirring rate of 110 rpm in a temperature controlled shaker. Likewise, the cells were sub-cultured to desired culture volumes of densities 5×10^6 cells/ml and viability of >95%. Subsequently, the cells were induced using sterile 500 µM copper sulfate and left in suspension for 7 days. After 7 days, the induced cells with medium were collected and samples were prepared as described earlier in the method section. The growth media was thoroughly dialyzed as described earlier to remove the copper ions as they can inhibit the binding of 6 × His-Tag *SRCR*s onto affinity column (Amersham Biosciences) during purification. The supernatant from the growth media was filtered through 0.22 µm filter and loaded on to the 3 × Histrap™ 5 ml column after diluting to double the original volume with buffer A (50 mM Tris, pH 8.0, 500 mM NaCl, 1% glycerol). After the sample was loaded onto the column, it was first washed (Wash I) with five column volumes (CV) of buffer A and then further washed (Wash II) with five CV of buffer A containing 25 mM imidazole following which a 25-300 mM imidazole gradient elution at flow rate of 5 ml/minute was carried out on an AKTA purifier (GE Healthcare Inc). Appropriate fractions identified from SDS-PAGE gels containing *SRCR*₁ and *SRCR*₁₂₃ were pooled and dialyzed overnight in 50 mM Tris pH 8.0, 1 mM EDTA, 50 mM NaCl. This sample was then bound to the Mono Q HR10/10 GL column (separates proteins based on their charges) and was eluted over a 0-30% of 1 M NaCl gradient at a flow rate of 3 ml/minute. Purified fractions identified from SDS-PAGE gels were pooled and concentrated to a final volume of 5 ml using a nitrogen pressurized (55 psi) Amicon stirred cell (EMD Millipore), fitted with 3000 Da MWCO membrane. As a final polishing step, the concentrated *SRCR*s were finally purified over a Superdex 200 10/300 GL column.

Western Blot

Western blots for SRCR₁ and SRCR₁₂₃ were carried out by dissolving the samples (2 µg/ml) in a non-reducing sample solubilizing buffer devoid of 2% β-mercaptoethanol and were electrophoretically separated on 12.5% SDS-PAGE gel and transferred onto polyvinylidene fluoride (PVDF) membranes using Semi-dry Western blot transfer system (BioRad, Inc) for 45 minutes at 15 Volts. These membranes were then washed with PBST (PBS containing 0.05% Tween-20) and blocked with 5% non-fat dry milk for 2 hours at room temperature (RT). Thereafter, membranes were washed three times in PBST (15 minutes each), and incubated with mouse anti-gp340 antibody (1:500, 213-06 Abcam) at 4 °C overnight. Subsequently, the membranes were incubated with the secondary antibody Horse Radish Peroxidase (HRP) conjugated goat anti-mouse antibody (1:3000) for 60 minutes at RT, washed and the results were recorded on a film using Enhanced Chemiluminescent (ECL) Plus (Pierce, Inc).

MALDI-TOF MS

MALDI-TOF studies were carried out according to procedures described earlier [26]. Briefly tryptic digests of the SRCR₁ and SRCR₁₂₃ peptides were eluted using 75% acetonitrile, 0.1% trifluoroacetic acid from ZipTips, airdried and reconstituted in matrix solution containing α-cyano-4-hydroxycinnamic acid dissolved in 50% acetonitrile, 0.1% trifluoroacetic acid and analyzed with a PerSeptive Voyager-DE MALDI-TOF mass spectrometer (PerSeptive Biosystems, Framingham, MA) [27]. Later, these peptides were laser-evaporated at 337 nm, and each spectrum was the cumulative average of 50–100 laser pulses. All peptides were measured as monoisotopic masses, and a trypsin autolytic peak at 2,164.1 *m/z* was selected for internal calibration. Up to one missed trypsin cleavage was allowed, although most matches did not contain any missed cleavages. This procedure resulted in mass accuracies of 100 ppm.

Expression and Purification of *S. mutans* full length AgI/II

S. mutans full length AgI/II (AgI/II) used in this study was prepared as described previously [6]. *E. Coli* BL21 (Δ*DE3*) bacterial cells harboring *AgI/II* gene were grown in 1 L Luria-Bertani (LB) medium at 37 °C. When cultures reached OD₆₀₀ of 0.6, expression was induced with 1 mM IPTG at 30 °C for 4 hours. Cells were harvested by centrifugation at 5000 × *g* and then resuspended in 50 mM Tris pH 8.0, 300 mM NaCl with one complete protease inhibitor tablet (Roche, Inc). Bacteria were lysed by sonication, and cell debris was cleared by centrifugation at 35,000 rpm for 1 hour using the Optima-L ultracentrifuge (Beckman, Inc). Supernatants [28] were filtered through a 0.22 µm filter, and AgI/II was purified by affinity, ion exchange, and size exclusion chromatography columns (Histrap™ 5 ml, Mono Q HR10/10 and Superdex 200 10/300 GL) as previously described for SRCRs.

Surface Plasmon Resonance

Real time binding analyses of the SRCR domains with AgI/II were analyzed using the BIAcore 2000 system (GE Healthcare) similar to earlier reported studies [5, 28]. The SRCR₁ and SRCR₁₂₃ were immobilized on the CM5 sensor chip through amine coupling of 0.4 M 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and 0.1 M N-hydroxysuccinimide (NHS) (1:1) upto ~3000 RU (resonance units). The unreacted residual N-hydroxy-succinimide esters were then inactivated using 1 M ethanolamine. AgI/II was then flowed over the chip surface at 2 µM concentration in binding buffer (20 mM HEPES, 2.5 mM CaCl₂ and 150 mM NaCl) at a flow rate of 20 µl/minute at 25 °C. After each experiment with the AgI/II, regeneration of the surface was accomplished using solution containing 1 M NaCl and 20 mM EDTA. The experiment was carried out in triplicates and the kinetics of

the association (K_A) and dissociation (K_D) rate constants were deduced using the 1:1 Langmuir Kinetic model on the BIA-Evaluation software.

Results and Discussion

Cloning of SRCR domains in pMT/V5-HisA vector

DNA Sequencing of the PCR products SRCR₁ and SRCR₁₂₃ ligated in the pMT/V5-HisA vector confirmed the presence of the *SRCR* genes in-frame with the pMT/V5-HisA vector. These recombinant vectors were then stably transfected into S2 cells and subsequently RT-PCR was carried out to measure the transcription ability and the level of gene expression. On the agarose gel electrophoresis the single band observed on lane 2 at ~400 bp corresponds to *SRCR*₁. However, on lane 3 along with the band at ~1200 bp that corresponds to *SRCR*₁₂₃ two other non-specific bands were also observed at ~400 bp and ~800 bp (Figure 2). After confirming the gene expression of the SRCRs, the stably transfected S2 cells were then selected and further expanded for large scale expressions.

Expression of SRCRs in pMT/V5-HisA vector

Our initial attempts to express the SRCR₁ and SRCR₁₂₃ from stably transfected S2 cells with recombinant pMT/V5-HisA vector did not yield SRCRs from the cell lysate nor the growth media. Although, the RNA was being produced, the absence of SRCRs expression clearly indicated that codon optimization was necessary for optimal expression of SRCR domains of gp340, which is of human origin in the *Drosophila* expression system.

Large scale expression and purification of SRCR domains

The optimized *SRCR* genes cloned into the pMT/BiP/V5-HisA vector (Figure 3A, B) was transfected stably into S2 cells, where RT-PCR (Figure 4) experiments displayed bands at ~400 bp and ~1200 bp, thereby confirming the stable transfection of optimized SRCR₁ and SRCR₁₂₃ respectively into the cell genome. These stably transfected cells harboring plasmids containing *SRCR*₁ and *SRCR*₁₂₃ genes were independently expanded and induced for expression. The growth media for SRCR₁ and SRCR₁₂₃ purified based over a 3 × HisTrap™ 5 ml column contained eluted fractions (Figure 5A, 6A) electrophoretically separated on an SDS-PAGE at ~15 kDa and at ~43 kDa corresponding to SRCR₁ and SRCR₁₂₃ (Figure 5B, 6B). The SRCRs were further purified on ion exchange column followed by size exclusion column (Figure 5C, 6C). Recovery of SRCRs on any size exclusion matrix was limited to 40%, and indicated that the SRCR domains adhered to both superose and sepharose matrices. The highly purified SRCR₁ and SRCR₁₂₃ obtained from one liter of growth media were ~30 mg and ~16 mg respectively, and each were concentrated to ~10 mg/ml (above which they aggregated). Western blots probed with the conformational dependent gp340 antibody (213-06, Abcam) confirmed these bands as SRCR₁ and SRCR₁₂₃ respectively (Figure 7). Further confirmation of the sequence was done through MALDI-TOF studies, where the appropriate peptide sequences shown in Table 1 were identified through a Basic Local Alignment Search Tool (BLAST). Finally, the functional adherence of the expressed SRCR₁ and SRCR₁₂₃ with AgI/II (Figure 8) was confirmed using surface plasmon resonance analysis [29, 30]. Evaluation of the affinity constants of AgI/II with SRCR₁ and SRCR₁₂₃ using 1:1 Langmuir binding model displayed K_D values of 2.31×10^{-8} M and 1.48×10^{-8} M respectively displaying strong binding interactions. Taken together, the codon optimization and usage of pMT/BiP/V5-HisA DES inducible/secretory vector significantly enhanced the SRCRs production.

Conclusions

The cloning and high level expression of functional human gp340's SRCR domains was successfully attained using *Drosophila* S2 cells. Although, initially the expression of SRCRs was limited by divergent codon usage, the problem was effectively overcome by optimizing the codons of SRCRs which are of human origin to suit the insect cell expression system. Additionally, the usage of inducible/secretory DES pMT/BiP/V5-HisA vector also enhanced the large scale SRCRs expression and purification. To-date, studies on the interaction of different bacterial strains and gp340 have been carried out with extracts from crude saliva, and the results from this study will allow for quantification of interaction between bacteria and gp340's highly purified recombinant SRCR domains. The cloning and purification strategy opted in this study can be further extrapolated to express and study the functional properties of other gp340 domains such as the CUB and ZP domains.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- SRCR domains of gp340 were successfully cloned with DES pMT/BiP/V5-HisA.
- Codon optimization enhanced protein production.
- Expressed SRCRs are folded properly and functionally adhere to *S. mutans* AgI/II.

Figure 1.
Primary sequence layout of Gp340 which contains fourteen SRCR domains, two CUB domains and one ZP domain.

Figure 2. Expression of *SRCR* genes in stably transfected S2 cells
RT-PCR of *SRCR*s recombinantly cloned in *Drosophila* expression vector pMT/V5-HisA. Lane 1) Generuler 1 kb plus DNA Ladder (Fermentas), Lane 2) RT-PCR product of *SRCR*₁ transfected S2 cells, Lane 3) RT-PCR product of *SRCR*₁₂₃ transfected S2 cells.

Figure 3. Plasmid constructs map showing the elements and schematic representations of the gene constructs (A) *SRCR₁* and (B) *SRCR₁₂₃*.

Figure 4. Expression of codon-optimized *SRCR* genes in stably transfected S2 cells
(A) RT-PCR of SRCRs recombinantly cloned in *Drosophila* expression vector pMT/BiP/V5-HisA. Lane 1) Generuler 1 kb plus DNA Ladder (Fermentas), Lane 2) RT-PCR product of SRCR₁₂₃ transfected S2 cells, Lane 3) RT-PCR product of SRCR₁ transfected S2 cells.

Figure 5. Purification of codon-optimized SRCR₁

A) Affinity chromatogram of SRCR₁ over the 3 × HisTrap™ 5 ml column [Milli absorbance unit (mAU) = A₂₈₀, CV = Column volume, % B = gradient elution]. B) Ladder: Page Ruler™ pre-stained protein ladder (Fermentas), Lanes 1-10: Eluted SRCR₁ peak fractions (5 ml each) from affinity purification and electrophoretically separated on a 12.5% SDS-PAGE gel C) 12.5% SDS-PAGE gels displaying SRCR₁ purified over Mono Q HR10/10 and Superdex 200 10/300 GL columns.

Figure 6. Purification of codon-optimized SRCR₁₂₃

A) Affinity chromatogram of SRCR₁₂₃ separated over a 3 × Histrap™ 5 ml column [Milli absorbance unit (mAU) = A₂₈₀, CV = Column volume, %B = gradient elution]. B) Ladder: Page Ruler™ pre-stained protein ladder (Fermentas), Lanes 1-7: Eluted SRCR₁₂₃ peak fractions (5 ml each) from affinity purification and electrophoretically separated on a 12.5% SDS-PAGE gel. C) 10% SDS-PAGE gels displaying SRCR₁₂₃ purified over Mono Q HR10/10 and Superdex 200 10/300 GL columns.

Figure 7. Immunoblot of SRCRs probed using the SAG mAb 213-06 confirmed the expression of SRCR₁ and SRCR₁₂₃.

Figure 8. Sensorgrams from surface plasmon resonance studies showing interaction of AgI/II with immobilized (A) SRCR₁ and (B) SRCR₁₂₃ on CM5 sensor chip. Adherence to labeled chip was measured in resonance unit (RU), where 1 RU = 1 picogram of protein.

Table 1

Constructs	Start -End	Observed	Nominal Mass (expected)	Nominal Mass (calculated)	Delta	Miss	Sequence	Ions Score
SRCR ₁	121 - 141	805.89	2414.65	2412.97	1.68	0	R.GSWGTVCDSDSWDTNDANVVCR.Q	43
	121 - 141	805.39	2413.15	2412.97	0.18	0	R.GSWGTVCDSDSWDTNDANVVCR.Q	47
SRCR ₁₂₃	219 - 235	870.53	1739.05	1738.91	0.14	0	R.ISPPVPTEGSESSLALR.L	72
	403 - 417	773.40	1544.80	1544.72	0.08	0	R.QLGCGWATSAPGNAR.F	16