Fish & Shellfish Immunology 34 (2013) 810–818

Contents lists available at SciVerse ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

# Identification and characterization of a cell surface scavenger receptor cysteine-rich protein of *Sciaenops ocellatus*: Bacterial interaction and its dependence on the conserved structural features of the SRCR domain

Reng Qiu [a,b], Bo-guang Sun [a], Jun Li [a,c], Xiao Liu [a], Li Sun [a,*]

[a] Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, 7 Nanhai Road, Qingdao 266071, PR China
[b] Graduate University of the Chinese Academy of Sciences, Beijing 100049, PR China
[c] School of Biological Sciences, Lake Superior State University, Sault Ste Marie, MI, USA

ARTICLE INFO

*Article history:*
Received 17 September 2012
Received in revised form
11 December 2012
Accepted 11 December 2012
Available online 2 January 2013

*Keywords:*
Scavenger receptor
*Sciaenops ocellatus*
Pattern recognition receptor

ABSTRACT

The scavenger receptor cysteine-rich (SRCR) proteins are secreted or membrane-bound receptors with one or multiple SRCR domains. Members of the SRCR superfamily are known to have diverse functions that include pathogen recognition and immunoregulation. In teleost, although protein sequences with SRCR structure have been identified in some species, very little functional investigation has been carried out. In this study, we identified and characterized a teleost SRCR protein from red drum *Sciaenops ocellatus*. The protein was named *S. ocellatus* SRCR1 (SoSRCRP1). SoSRCRP1 is 410-residue in length and was predicted to be a transmembrane protein, with the extracellular region containing a collagen triple helix repeat and a SRCR domain. The SRCR domain has six conserved cysteines, of which, C338 and C399, C351 and C409, and C379 and C389 were predicted to form three disulfide bonds. *SoSRCRP1* expression was detected mainly in immune-relevant tissues and upregulated by bacterial and viral infection. In head kidney leukocytes, bacterial infection stimulated the expression of *SoSRCRP1*, and the expressed SoSRCRP1 was localized on cell surface. Recombinant SoSRCRP1 (rSoSRCRP1) corresponding to the SRCR domain was purified from *Escherichia coli* and found to be able to bind Gram-negative and Gram-positive bacteria. To examine the structure–function relationship of SoSRCRP1, the mutant proteins SoSRCRP1M1, SoSRCRP1M2, SoSRCRP1M3, and SoSRCRP1M4 were created, which bear C351S and C409S, C338S, C379S, and R325A mutations respectively. Compared to rSoSRCRP1, all mutants were significantly reduced in the ability of bacterial interaction, with the highest reduction observed with SoSRCRP1M4. Taken together, these results indicate that SoSRCRP1 is a cell surface-localized SRCR protein that binds bacterial ligands in a manner that depends on the conserved structural features of the SRCR domain.


## 1. Introduction

Scavenger receptors (SR) are a group of proteins that recognize and uptake polyanionic macromolecules, such as modified low-density lipoproteins (LDL), apoptotic cells, and bacteria, by receptor-mediated endocytosis. Scavenger receptors are classified into eight classes named class A–class H [1]. The class A scavenger receptors include SR-A, macrophage receptor with collagenous structure (MARCO), and scavenger receptor with C-type lectin (SRCL) [2]. SR-A was the first scavenger receptor cysteine-rich (SRCR) protein identified at sequence level. It is a type II transmembrane glycoprotein that has three isoforms, i.e. SR-AI, SR-AII, and SR-AIII. SR-AI is structurally composed of six motifs: an N-terminal cytoplasmic domain, a transmembrane region, a spacer region, an α-helical coiled coil motif, a collagenous domain, and a C-terminal SRCR region [3]. MARCO is similar to SR-AI in structure, except that it has a longer collagenous domain and lacks the α-helical coiled coil domain [4,5].

Structurally, the SRCR domains are 90–110 amino acid residues in length and contain six conserved cysteine residues that form three intra-domain disulfide bonds. Based on their structural differences, the SRCR domains are divided into two groups named A and B. Group A domains contain six cysteine residues, while group B domains contain eight cysteine residues [6]. MARCO is one

* Corresponding author. Tel./fax: +86 532 82898829.
*E-mail addresses:* lsun@qdio.ac.cn, lsun@ms.qdio.ac.cn (L. Sun).

1050-4648/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fsi.2012.12.016

of the most extensively studied SRCR proteins with group A SRCR domain. Crystal structure analysis of the monomeric and dimeric forms of MARCO SRCR showed that the monomer forms a compact globular fold with a twisted five-stranded antiparallel beta-sheet and a long loop covering a single alpha-helix, while the dimer is formed via beta-strand swapping of two monomers and contains a large eight-stranded beta-sheet [7]. A basic cluster and an acidic cluster are located in the beta-sheet region and the long loop region respectively, and both clusters were found to be involved in ligand binding [7,8].

To date more than 30 different members of the SRCR family have been identified, mostly in mammals. These proteins have one or multiple SRCR domains and in general exist in membrane-bound or soluble form [6]. Some SRCR members, e.g. SR-A and MARCO, have ligands that include surface components of Gram-negative and Gram-positive bacteria, such as lipopolysaccharide and lipoteichoic acid, and thus act as pattern recognition receptors (PRRs) and participate in innate as well as adaptive immune response [2,9,10]. MARCO is known to recognize and bind *Escherichia coli* and *Staphylococcus aureus*, while SR-A I/II is the major PRR for Group B Streptococcus and *Streptococcus pyogenes* [10–13]. The importance of SRCR in host immune defense has been demonstrated in animal studies, which showed that SR-A-deficient mice exhibit increased susceptibility to infections to *Listeria monocytogenes*, herpes simplex virus, and *S. aureus* [14,15].

In teleost, SRCR homologs have been identified in a few species, however, very little functional study has been carried out. As a result, the biological properties of fish SRCR proteins are unknown. In this report, we identified a SRCR protein from red drum (*Sciaenops ocellatus*) and named it *S. ocellatus* SRCR1 (SoSRCRP1). We found that SoSRCRP1 is a cell surface-localized protein with a bacterial binding capacity that depends on the structure of the SRCR domain. These findings provide a basis for studying the functionality of teleost SRCR.

## 2. Materials and methods

### 2.1. Fish

Red drum (*Sciaenops ocellatus*) were purchased from a commercial fish farm in Shandong Province, China and maintained at 22 °C in aerated seawater. Fish were acclimatized in the laboratory for two weeks before experimental manipulation and euthanized with tricaine methanesulfonate (Sigma, St. Louis, MO, USA) before tissue collection as reported previously [16].

### 2.2. Bacterial strains and culture conditions

The Gram-negative fish bacterial pathogens *Edwardsiella tarda* TX1 and *Vibrio/Listonella anguillarum* C312, and the Gram-positive fish bacterial pathogen *Streptococcus iniae* SF1 have been reported previously [17–19]. *E. coli* BL21(DE3) was purchased from Tiangen (Beijing, China). All strains were cultured in Luria–Bertani broth (LB) medium at 28 °C (for fish pathogens) or 37 °C (for *E. coli*).

### 2.3. Cloning of SoSRCRP1

A cDNA library of red drum head kidney (HK), spleen, and liver was constructed as reported previously [20]. Plasmid was isolated from 1200 clones and subjected to DNA sequence analysis; one clone was found to contain the cDNA of *SoSRCRP1* with 5′- and 3′-untranslated regions (UTRs). The nucleotide sequence of *SoSRCRP1* has been deposited in GenBank database under the accession number JX678713.

### 2.4. Sequence analysis

The cDNA and amino acid sequences of SoSRCRP1 were analyzed using the BLAST program at the National Center for Biotechnology Information (NCBI) and the Expert Protein Analysis System. Domain search was performed with the simple modular architecture research tool (SMART) version 4.0 and the conserved domain search program of NCBI. The molecular mass and theoretical isoelectric point (pI) were predicted using EditSeq in DNASTAR software package (DNASTAR Inc. Madison, WI, USA). Multiple sequence alignment was created with the ClustalX program. Subcellular localization prediction was performed with WoLF PSORT.

### 2.5. Plasmid construction

The primers used for plasmid construction are listed in Table 1. To construct pEtSRCRP1, which expresses a His-tagged protein corresponding to the SRCR domain of SoSRCRP1, the coding sequence of the SRCR region was amplified by PCR with primers F1 and R1 (Table 1); the PCR products were ligated with the T–A cloning vector T-Simple (TransGen Biotech, Beijing, China), and the recombinant plasmid was digested with EcoRV to retrieve the *SoSRCRP1*-containing fragment, which was inserted into pET259 [21] at the SwaI site, resulting in pEtSRCRP1. pEtSRCRP1M1, which expresses the mutant protein SoSRCRP1M1 bearing C351S and C409S mutation, was constructed by overlapping PCR as follows: the first PCR was performed with the primers F1 and M1R1, the second PCR was performed with the primers M1F1 and M1R2, and the fusion PCR was performed with the primer pair F1/M1R2. The PCR products were digested with EcoRV and inserted into pET259 as described above, resulting in pEtSRCRP1M1. pEtSRCRP1M2, which expresses SoSRCRP1M2 bearing C338S mutation, was constructed as described above, except that the first PCR was performed with the primers F1 and M2R1, the second PCR was performed with the primers M2F1 and R1, and the fusion PCR was performed with the primer pair F1/R1. pEtSRCRP1M3, which expresses SoSRCRP1M3 bearing C379S mutation, was constructed as described above, except that the first PCR was performed with the primers F1 and M3R1, the second PCR was performed with the primers M3F1 and R1, and the fusion PCR was performed with the primer pair F1/R1. pEtSRCRP1M4, which expresses SoSRCRP1M4 bearing R325A mutation, was constructed as described above, except that the first PCR was performed with the primers F1 and M4R1, the second PCR was performed with the primers M4F1 and R1, and the fusion PCR was performed with the primer pair F1/R1.

**Table 1**
Primers used in this study.

| Primer | Sequences (5′ → 3′)[a] |
|---|---|
| F1 | GATATCATGGTGCGTCTCGTGCC (EcoRV) |
| R1 | GATATCGGCGCACTGCACTCCG (EcoRV) |
| M1F1 | AGGTGATCAGCAGGATGCTG |
| M1R1 | CCTGCTGATCACCTTGCC |
| M1R2 | GATATCGGCGCTCTGCACTCCG (EcoRV) |
| M2F1 | CACCGTGAGCGACGACAACTTT |
| M2R1 | CGTCGCTCACGGTGCCCC |
| M3F1 | TGCGAAGCACAGGAACAGAGT |
| M3R1 | CTCTGTTCCTGTGCTTCGCAGA |
| M4F1 | GCGCGGTGGAGGTGAAGGT |
| M4R1 | TCACCTCCACCGCGCCTC |
| RTF1 | GACGACAACTTTGACACTCTGG |
| RTR1 | ACATCCGACTCTGTTCCTGTG |

[a] Underlined nucleotides are restriction sites of the enzymes indicated in the brackets at the ends.