Microbiological Research 169 (2014) 824–834

Contents lists available at ScienceDirect

Microbiological Research

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

Molecular characterization of a novel proto-type antimicrobial protein galectin-1 from striped murrel

Abirami Arasu[a,b], Venkatesh Kumaresan[a], Akila Sathyamoorthi[a,c], Mukesh Kumar Chaurasia[a], Prasanth Bhatt[a], Annie J. Gnanam[d], Rajesh Palanisamy[a], Kasi Marimuthu[e], Mukesh Pasupuleti[f], Jesu Arockiaraj[a,*]

[a] Division of Fisheries Biotechnology & Molecular Biology, Department of Biotechnology, Faculty of Science and Humanities, SRM University, Kattankulathur 603203, Chennai, Tamil Nadu, India
[b] Department of Microbiology, SRM Arts & Science College, Kattankulathur 603203, Chennai, Tamil Nadu, India
[c] Department of Biotechnology, SRM Arts & Science College, Kattankulathur 603203, Chennai, India
[d] Institute for Cellular and Molecular Biology, The University of Texas at Austin, 1 University Station A4800, Austin, TX 78712, USA
[e] Department of Biotechnology, Faculty of Applied Sciences, AIMST University, Semeling Bedong, 08100 Bedong, Kedah, Malaysia
[f] Lab PCN 206, Microbiology Division, CSIR-Central Drug Research Institute, B.S. 10/1, Sector 10, Jankipuram Extension, Sitapur Road, Lucknow 226031, Uttar Pradesh, India

ARTICLE INFO

Article history:
Received 24 January 2014
Received in revised form 21 March 2014
Accepted 27 March 2014
Available online 4 April 2014

Keywords:
Galectin
Murrel
Gene expression
Erythrocytes agglutination
Bacterial agglutination

ABSTRACT

In this study, we reported a molecular characterization of a novel proto-type galectin-1 from the striped murrel *Channa striatus* (named as CsGal-1). The full length CsGal-1 was identified from an established striped murrel cDNA library and further we confirmed the sequence by cloning. The complete cDNA sequence of *Cs*Gal-1 is 590 base pairs (bp) in length and its coding region encoded a poly peptide of 135 amino acids. The polypeptide contains a galactoside binding lectin domain at 4–135. The domain carries a sugar binding site at 45–74 along with its signatures (H^{45}-X-Asn^{47}-X-Arg^{49} and Trp^{69}-X-X-Glu^{72}-X-Arg^{74}). *Cs*Gal-1 shares a highly conserved carbohydrate recognition domain (CRD) with galectin-1 from other proto-type galectin of teleosts. The mRNA expressions of *Cs*Gal-1 in healthy and various immune stimulants including *Aphanomyces invadans*, *Aeromonas hydrophila*, *Escherchia coli* lipopolysaccharide and poly I:C injected tissues of *C. striatus* were examined using qRT-PCR. *Cs*Gal-1 mRNA is highly expressed in kidney and is up-regulated with different immune stimulants at various time points. To understand its biological activity, the coding region of *Cs*Gal-1 gene was expressed in an *E. coli* BL21 (DE3) cloning system and its recombinant protein was purified. The recombinant *Cs*Gal-1 protein was agglutinated with mouse erythrocytes at a concentration of 4 μg/mL in a calcium independent manner. *Cs*Gal-1 activity was inhibited by D-galactose at 25 mM^{-1} and D-glucose and D-fructose at 100 mM^{-1}. The results of microbial binding assay showed that the recombinant *Cs*Gal-1 protein agglutinated only with the Gram-negative bacteria. Interestingly, we observed no agglutination against Gram-positive bacteria. Overall, the study showed that *Cs*Gal-1 is an important immune gene involved in the recognition and elimination of pathogens in *C. striatus*.

1. Introduction

Lectins are proteins that bind to specific carbohydrate structure and can thus recognize particular glycoconjugates among the vast array expressed in animal tissues (Barondes et al. 1994a). Dutta et al. (2005) reported that lectins are ubiquitous in nature and due to their unique property of binding to carbohydrate moieties on cell surfaces, play an important role in cellular events like agglutination, proliferation, opsonization, signal transduction, metastasis and apoptosis. Lectins also have a significant role in the immune responses of the host. They bind specifically to the carbohydrate molecules expressed on the pathogens and help in their rapid clearance by enhancing opsonization and phagocytosis and also by increasing the oxidative burst activities (Saito et al. 1997; Tasumi et al. 2002; Janeway and Medzhitov 2002; Tateno et al. 2002; Nauta et al. 2004).

Lectins have so far been isolated from serum, plasma, skin mucus, egg surfaces and egg components of fish (Jensen et al. 1997;

* Corresponding author. Tel.: +91 44 27452270; fax: +91 44 27453903.
E-mail address: jesuaraj@hotmail.com (J. Arockiaraj).

http://dx.doi.org/10.1016/j.micres.2014.03.005
0944-5013/

Ottinger et al. 1999; Dong et al. 2004; Tasumi et al. 2004). Based on the structures and functions, the lectins are classified into extracellular lectins and intracellular lectins. The extracellular lectins are ficolins, chitinase-like lectins, intelectins, C, R, S (galectins) and I (siglecs) type lectins. The intracellular lectins are calnexin, pentraxins, M, L, P, R type lectins and F-box lectins (Barondes et al. 1994a,b; Drickamer and Taylor, 1998). Among these, galectins comprise one of the major families that have several characteristics in common: Ca^{2+} independent activity, specificity for β-galactosides, a blocked N-terminus, lack of a signal peptide, lack of attached carbohydrate, and predominantly cytoplasmic location (Sharon 1993). Additionally, many galectins possess cysteine residues existing as free thiols, and are often unstable under oxidizing conditions (Tasumi et al. 2004).

Galectins were formerly known as S-type or S-Lac lectins (Barondes et al. 1994b). The first protein discovered in the family of galectin was galectin-1 (Camby et al. 2006). Galectins are a phylogenetically conserved lectin superfamily defined in 1994 as a shared consensus of amino acid sequences of ~136 amino acids and the carbohydrate recognition domain (CRD) responsible for β-galactoside binding (Barondes et al. 1994a). Based on the structure and number of CRD, galectins have been classified into three types namely proto, chimera and tandem-repeat (Vasta 2012). The proto type possesses one CRD and is non-covalently linked homodimer. The chimera type contains a C-terminal CRD and a N-terminal domain rich in proline and glycine. The tandem-repeat consists of two CRDs which are joined by a functional linker peptide. The galectins are found in species ranging from sponges to man. So far, 15 mammalian galectins have been reported (Camby et al. 2006). However, only limited work have been published about fish galectin to date of which a few are listed: A prototype galectin (named as Congerin) has been reported from the skin mucus of Japanese conger, *Conger myriaster* (Kamiya et al. 1988; Nakamura et al. 2006) and it has two isotypes, namely, Congerin I and II (Muramoto and Kamiya 1992; Muramoto et al. 1999; Ogawa et al. 1999; Nakamura et al. 2006). Tasumi et al. (2002) reported a galectin from the skin mucus of Japanese eel *Anguilla japonica* and it exhibited β-galactoside specific activity in a Ca^{2+} independent manner. Another α-methyl galactose binding lectin from the Indian catfish, *Clarias batrachus* have been reported by Dutta et al. (2005). Additionally, a lower vertebrate African clawed frog, *Xenopus laevis* (Marshal et al. 1992) also possessed galectin in the skin mucus.

The available literature (Camby et al. 2006) report that many galectins contain one CRD and are biologically active as monomers (galectin 5, 7 and 10), homodimers (galectin 1, 2, 11, 13, 14 and 15) or oligomers (galectin 3) that aggregate through their non-lectin domain. Some other galectins (galectin 4, 6, 8, 9 and 12) contain two CRDs which are connected by a short linker peptide. The CRDs of all the galectins share an affinity for the minimum saccharide ligand N-acetyl lactosamine. It is a common disaccharide present in many cellular glycoproteins. Galectins can also recognize different modifications of this minimum saccharide ligand, thus demonstrating the fine specificity of certain galectins for tissue or developmentally specific ligands (Ahmad et al. 2004). Hughes (2001) reported that many galectins do not have a signal sequence which is required for protein secretion through the usual secretary pathway, however, some galectins are secretory proteins which are present in the extracellular components (Hughes 2001). The intracellular activity of galectin-1 is independent while its extracellular activity is mainly dependent on its lectin activity.

Galectins, a family of animal lectins with affinity for β-galactosides, can form multivalent complexes with cell surface glycoconjugates and deliver a variety of intracellular signals to modulate cell activation, differentiation and survival. Recent efforts involving genetic or biochemical manipulation of O- and N-glycosylation pathways, as well as blockade of the synthesis of endogenous galectins, have illuminated essential roles for galectin-glycoprotein lattices in the control of biological processes including receptor turnover, endocytosis, host–pathogen interactions, immune cell activation and homeostasis (Rabinovich et al. 2007). As many as fifteen galectins have been identified and proposed to mediate diverse biological processes involved in the regulation of innate and adaptive immune responses, such as cell activation, differentiation, cytokine secretion and apoptosis (Brewer et al. 2002; Toscano et al. 2007).

The immunosuppressive and anti-inflammatory activity of galectin-1 has been well established by *in vivo* and *in vitro* studies in various animals (Kiss et al. 2007; Rajan et al. 2013; Liu et al. 2013). Moreover, it has been also shown that galectin-1 inhibits chemotaxis and trans-endothelial migration of polymorphonuclear leukocytes *in vitro* (Brewer et al. 2002). Galectin-1 is also involved in mediating cell adhesion, eliciting signaling and forming lattices. Its ligands are one of the master regulators of immune responses such as T-cell homeostasis and survival, T-cell immune disorders, inflammation and allergies as well as host-pathogen interactions (Rabinovich et al. 2007).

Striped murrel *Channa striatus* is a popular food-fish from South and South East Asia, because of its delicious and tasty flesh. Epizootic ulcerative syndrome (EUS), a fungal (*Aphanomyces invadans*) and bacterial (*Aeromonas hydrophila*) causing disease has disrupted the murrel culture system (Arockiaraj et al. 2003). Efforts have been made to prevent and control the disease by chemotherapeutics, herbal immunostimulants, vaccination, *etc*. However, the defense mechanisms are still poorly understood in murrel. Therefore, identification of immune related gene and their expression studies are imperative.

So far, nearly 1000 research papers have reported about galectins in PubMed and according to our knowledge, there is no information about gene characterization or gene expression of *C. striatus* galectin-1 (named as *Cs*Gal-1). In this article, we have reported *Cs*Gal-1 cDNA full length sequence, identified from the constructed murrel cDNA library genome sequence FLX^{TM} (GS-FLX^{TM}) technique and characterized bioinformatically. The transcriptional differentiation of *Cs*Gal-1 mRNA have been compared using fungus (*A. invadans*), bacteria (*A. hydrophila*), *Escherichia coli* lipopolysaccharide (*E. coli* LPS) and poly I:C [polyinosinic-polycytidylic acid sodium salt, a synthetic analog of double-stranded RNA (dsRNA), a molecular pattern associated with viral infection. It is composed of a strand of poly(I) annealed to a strand of poly(C)] immune-stimulants. Furthermore, overexpression and purification of recombinant protein were conducted using *E. coli* BL21 (DE3) bacterial expression system to study its biological activities.

2. Materials and methods

2.1. Experimental fish

Healthy *C. striatus* (average body weight of 50 g) were obtained from the Surya Agro Farms, Erode, Tamil Nadu, India. The fishes were transported to Division of Fisheries Biotechnology & Molecular Biology, SRM University in oxygenated polythene bags. They were maintained in 15 flat-bottomed plastic containers (150 L) with aerated and filtered de-chlorinated freshwater (water quality: dissolved oxygen, 5.8 ± 0.2 mg/L; water temperature, 28 ± 1 °C and pH, 7.2 ± 0.2) in the laboratory. All fishes were acclimatized for 1 week before being challenged to various immune stimulants. A maximum of 15 fishes per tank were maintained during the experiment. During acclimatization period, the fishes were fed *ad libitum* two times daily at 09.00 and 16.00 h with a commercially available fish feed (Cargill Animal Nutrition, Andhra Pradesh, India).