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Antimicrobial response of galectin-1 from rock bream *Oplegnathus fasciatus*: Molecular, transcriptional, and biological characterization

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ABSTRACT

In this study, we describe the identification and characterization of a proto type galectin, galectin-1, from rock bream *Oplegnathus fasciatus* (OfGal-1). Galectins are evolutionarily conserved carbohydrate binding lectins that show a wide range of functions related to development and immune physiology. They have been identified as pattern recognition receptors of innate immune system that recognize a broad range of microbes. OfGal-1 cDNA comprised of 993 bp with an open reading frame of 408 bp that encodes 135 amino acids. A single carbohydrate recognition domain was present in the OfGal-1 amino acid sequence. The sequence comparison by multiple and pairwise alignments and the phylogenetic tree emphasized the strong evolutionary conservation of Gal-1. The typical β-sandwich structure was identified from the predicted tertiary structure. The constitutive expression of mRNA transcripts was detected in a wide range of tissues examined, with the highest expression in the heart. Immune challenges with live bacteria (*Edwardsiella tarda* and *Streptococcus iniae*), rock bream irido virus, and mitogens (lipopolysaccharide and poly I:C) modulated the expression of *OfGal-1* mRNAs in the gills, head kidney, and liver. The recombinant OfGal-1 (rOfGal-1) strongly agglutinatinated the human erythrocytes, and this hemagglutination was inhibited by lactose and D-galactose. A wide range of bacteria (*S. iniae, S. parauberis, Escherichia coli, Edwardsiella tarda, Vibrio anguillarum, Vibrio harveyi,* and *Vibrio tapetis*) and a ciliate (*Miamiensis avidus*) were also effectively recognized by rOfGal-1. Significant antiviral activity against rock bream irido virus was also demonstrated by rOfGal-1. Collectively, results from the present study indicate that OfGal-1 can recognize a wide range of microbes and is a vital pattern recognition receptor in the innate immune system of rock bream.

1. Introduction

Pathogen recognition via protein-carbohydrate interactions is mainly mediated by soluble and cell-associated lectins [1]. Lectins are known to play a significant role in cellular functions, including agglutination, proliferation, opsonization, phagocytosis, signal transduction, metastasis, and apoptosis [2–4]. Based on their sequence of carbohydrate recognition domains (CRD), animal lectins have been classified as C-type, F-type, P-type, ficolins, pentraxins, and galectins [5–7].

In particular, galectins constitute a family of evolutionarily conserved, soluble, Ca^{2+}-independent lectins. They are characterized by their affinity for β-galactoside and are ubiquitous in eukaryotes, including fungi, poriferans, protostomes, and deuterostomes [8,9]. Although galectins lack a signal peptide, they are synthesized in the cytoplasm and transported to the extracellular surface through non-classical pathways or function in the cytoplasm and/or translocate to the nucleus [10]. About 15 different galectin subfamilies have been characterized in mammals, and these are further classified as proto, chimera, and tandem-repeat types based on their molecular structural variations [8,11]. The prototype galectins consist of a single CRD (galectin-1, −2, −5, −7, −10, −11, −13, −14, and −15), the chimera type

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consists of a CRD (galectin-3) and a non-carbohydrate peptide chain. The tandem-repeat type, possess two CRDs (galectin-4, -8 and -9) linked by a linker peptide chain [5,12].

In earlier studies, galectins were reported to function as mediators in the developmental process by binding to endogenous glycans; however, recent studies have clearly demonstrated their wide range of roles in the innate and adaptive immunity. Galectins bind with the exogenous glycan-ligands, termed pathogen-associated molecular patterns (PAMPs), of microbial pathogens including bacteria, fungi, protozoan parasites and virus and function as pattern recognition receptors (PRRs) in the innate immune system [6,13]. In addition, mammalian galectins participate in acute and allergic inflammation and leukocyte infiltration, migration, and recruitment [14].

Galectin-1 plays several roles in development, innate and adaptive immunity, including adhesion, regulation of cellular proliferation and regulation of cell-survival [15]. In humans, it stimulates vascular cell proliferation [16]; however, while it inhibits fibroblast proliferation at high concentrations it is mitogenic at low concentrations [17]. It exhibits its anti-inflammatory effects by blocking the movement of hematopoietic cells, mature granulocytes, and monocytes, and inhibiting chemotaxis induced by stromal derive factor-1 [18]. Pro- or anti-apoptotic effects of galectin-1 that depend on the developmental stages [6] as well its role in antimicrobial activities in several vertebrates have been described [19–22].

Microbial recognition by galectins in several vertebrates, including fish, has been reported. Two skin mucus galectins from *Conger myriaster*, congerin I and II, agglutinated *Vibrio anguillarum* [23]. A prototype galectin from *Trachidermus fasciatus* was found to agglutinate and bind to both Gram positive and Gram negative bacteria [19]. Similarly, a prototype galectin from *Anguilla japonica* [24] and a galectin-1 from *Gadus morhua* [25] agglutinated pathogenic bacteria. Moreover, it has been found that *T. fasciatus* galectin exerts its antifungal effects via agglutination [19] and a proto type galectin from *C. myriaster* has anti-parasitic activity against a nematode parasite [26]. Furthermore, galectin-1 from *Paralichthys olivaceus* has been found to exhibit anti-viral activity against lymphocystis disease virus *in vivo* [27], and in a previous study, nodavirus infection in *Dicentrarchus labrax* significantly upregulated the expression of *galectin-1* [22].

Rock bream *Oplegnathus fasciatus* is one of the most important fish species in the mariculture of South Korea. Considering the decline in production, which is mainly due to infection by *Streptococcus iniae*, *Edwardsiella tarda,* and rock bream irido virus (RBIV) [28,29], the present study was conducted to understand the role of galectin-1 (termed *OfGal-1*) as a PRR in the defense mechanism of rock breams. Investigating the biological function of galectin-1 in *O. fasciatus* may provide new insights about its roles in the innate immunity as well as its evolutionary features at a molecular level.

2. Materials and methods

2.1. Experimental fish

Healthy rock bream fish (average body weight of 50 g) were obtained from the Jeju Special Self-Governing Province Ocean and Fisheries Research Institute, Jeju, Republic of Korea. They were maintained in 400-L flat-bottom tanks filled with aerated, sand-filtered sea water (salinity 34 ± 1‰, pH 7.6 ± 0.5) at 24 ± 1 °C. All fish were acclimatized to the laboratory conditions for 1 week prior to experiments. No clinical signs for disease were observed.

2.2. Tissue sampling from healthy fish

In order to examine the mRNA expression pattern of *OfGal-1* under normal physiological conditions, three naïve fish were dissected. Blood was withdrawn from the caudal vein using heparinized syringe and peripheral blood cells (PBCs) were separated from plasma by centrifugation (3000 × *g* for 10 min at 4 °C). Additionally, tissues including liver, skin, muscle, spleen, gill, kidney, brain, intestine, head kidney, and heart were obtained from dissected fish. All tissue samples were snap frozen in liquid nitrogen and stored in −80 °C for total RNA extraction and cDNA synthesis.

2.3. Tissue sampling from immune challenged fish

To determine the expression pattern of *OfGal-1* under experimentally induced immune stress conditions, the fish were divided into 7 groups and challenges were performed as described previously [30]. Two pathogen-derived mitogens [lipopolysaccharide (LPS) and polyiosinic:polycytidylic acid (poly I:C)], two pathogenic bacteria (*S. iniae* and *Edwardsiella tarda*), and a virus (rock bream iridovirus (RBIV)) were used and their concentrations were adjusted in phosphate buffered saline (PBS). LPS (125 μg in 100 μL), poly I:C (150 μg in 100 μL), *S. iniae* (1×10^7 CFU in 100 μL PBS), *E. tarda* (5×10^5 CFU in100 μL PBS) and 100 μL of PBS alone were administered intraperitoneally to the respective fish group, while 100 μL of RBIV in PBS (10^2 tissue culture infectious dose 50; $TCID_{50}$) was injected intramuscularly. The remaining fish were classified as unchallenged group. Fish were dissected at 3, 6, 12, 24 and 48 h post-injection (p.i.), and tissue samples from the gill, head kidney and liver were collected, snap frozen in liquid nitrogen, and stored at −80 °C for RNA isolation.

2.4. Sequence identification and characterization of OfGal-1

The cDNA containing complete sequence of *OfGal-1* was identified using the Basic Local Alignment Search Tool (BLAST) algorithm in NCBI (http://blast.ncbi.nlm.nih.gov/Blast) from a previously established cDNA library and was compared with other known *galectin* sequences. DNAssit (version 2.2) was used to determine the open reading frame (ORF) and amino acid sequences. The ORF of the *OfGal-1* was cloned into the T-Vector pMD20 (TaKaRa) using specific primers (Table 1) and the sequence was confirmed by Macrogen, Korea. The deduced amino acid sequence was analyzed by the Expert Protein Analysis System (http://www.expasy.org) and the presence of the signal peptide was examined by SignalP 4.0 (http://www.cbs.dtu.dk/services/SignalP). The DISULFIND program (http://disulfind.disi.unitn.it) was used to predict the cysteine disulfide bonds in the OfGal-1 sequence. To determine the putative conserved domains in the OfGal-1 predicted protein, PROSITE Scan (http://prosite.expasy.org/cgibin/prosite) was employed. Pairwise and multiple alignments were carried out by ClustalW method in the BioEdit Sequence alignment editor package. The three-dimensional protein structural homology model was generated by Swiss-Model (http://swissmodel.expasy.org) and annotated by PyMOl molecular graphic software version 1.3. The phylogenetic tree was reconstructed using MEGA 5.0 package (http://megasofteare.net). To deduce the confidence value for the phylogenetic analysis, bootstrap trials were replicated 5000 times.

2.5. Total RNA isolation and cDNA synthesis

Total RNA was extracted from frozen tissues using Tri Reagent™ (Sigma–Aldrich) by following manufacturer's instructions and the