Developmental and Comparative Immunology 65 (2016) 25–30

Contents lists available at ScienceDirect

Developmental and Comparative Immunology

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

Molecular cloning and expression analysis of a new lily-type lectin in the rock bream, *Oplegnathus fasciatus*

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ARTICLE INFO

Article history:
Received 10 May 2016
Received in revised form
16 June 2016
Accepted 19 June 2016
Available online 21 June 2016

Keywords:
Oplegnathus fasciatus
Lily-type lectin
Streptococcus iniae
Edwardsiella tarda
Red sea bream iridovirus

ABSTRACT

A new lily-type lectin RbLTL was identified from rock bream (*Oplegnathus fasciatus*) and its expression analysed. In this study, a new lily-type lectin gene (RbLTL) was cloned from rock bream using expressed sequence tag (EST) analysis. The full-length RbLTL cDNA was encoding a 117-amino acid protein. The deduced amino acid sequence of RbLTL contained all of the conserved features crucial for its fundamental structure, including B-lectin domain and three D-mannose binding sites. RbLTL mRNA was predominately expressed in the gills, with reduced expression noted in intestine tissue. Expression analysis of time series sampled fertilized eggs revealed that expression gradually increased 1, 3, 12, and 24 h: However, expression decreased at 36 h. RbLTL expression was differentially up-regulated in rock bream gills challenged with *Streptococcus iniae*, *Edwardsiella tarda* and RSIV. Our results revealed that novel rock bream lily-type lectin may be an important molecule involved in pattern recognition and pathogen elimination in the innate immunity of rock bream.

1. Introduction

Lectins are widely distributed from *Caenorhabditis elegans* to *Homo sapiens*. Lectins bind specifically and reversibly to carbohydrate structures, particularly the sugar moiety of glycoconjugates and glycoproteins (Dong et al., 2004). Lectins are a group of sugar-binding proteins that recognize specific carbohydrate structures and agglutinate various cells by binding to cell-surface glycoconjugates (Saraiva et al., 2011). The chemical properties of animal lectins, including sugar specificity, divalent ion requirement, and carbohydrate binding domain structure, are useful for the classification of these lectins into several families (Medzhitov and Janeway. 2002). Physiologically, animal lectins have been postulated to perform important roles in various endogenous biological processes, including self-defense and cell-cell recognition (Janeway, 1989).

According to their distinct structures and functions, lectins are classified as calnexin C-, L-, P-, I-, R- and S-types (Janeway and Medzhitov, 2002). In particular, fish lectins are mediators of non-self-recognition in a variety of biological processes (Sharon and Lis, 2004). Specifically, fish lectins identify and stimulate the uptake of pathogens by phagocytes, facilitate innate complement-mediated cell lysis and enhance natural killer cell activity (Hoffmann et al., 1999).

Fish lectins have been identified from rainbow trout (*Oncorhynchus mykiss*) (Zhang et al., 2000), carp (*Cyprinus carpio*) (Fujiki et al., 2001), and eel (*Anguilla japonica*) (Tasumi et al., 2002). Lectins that bind to specific sugars such as mannose (Ottinger et al., 1999; Konstantina and Ioannis, 2006), galactose (Vitved et al., 2000), fucose (Honda et al., 2000) and rhamnose (Okamoto et al., 2005) have also been isolated and characterized in fish.

Recently, a lily-type lectin sequence was identified in orange-spotted grouper (*Epinephelus coioides*), large yellow croaker (*Larimichthys crocea*), Bartail flathead (*Platycephalus indicus*), Spotnape ponyfish (*Leiognathus nuchalis*). Lectins interacting with yeast and fish bacterial pathogens have also been functionally characterized

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http://dx.doi.org/10.1016/j.dci.2016.06.014
0145-305X/

in the conger eel (*Conger myriaster*) (Tsutsui et al., 2007) and coho salmon (*Oncorhynchus kisutch*) (Yousif et al., 1994).

Vertebrates and invertebrates lack an adaptive immune system and mainly rely on innate immunity to resist pathogen invasion. How to distinguish self from non-self is a crucial problem of innate immunity (Locker et al., 2004). The gill associated lymphoid tissue developed into an immunologically active tissue made up of potent immune factors and immune reactive cells (Lazado and Caipang, 2014), such as lymphocytes, macrophages, eosinophilic granulocytes, neutrophils and antibody-secreting cells (ASC) (Lin et al., 1998; Dos santos et al., 2001; Mulero et al., 2008), and it possesses numerous immune-related molecules including antimicrobial peptides (Caipang et al., 2010; Ruangsri et al., 2014).

In this study, we describe the molecular identification and expression of the lily-type lectin gene in a teleost and subsequently assess the expression of this gene during the egg developmental stage as well as in response to injected pathogens. We are observed abundantly expression patterns that bacterial and viral infection at differential rimes, respectively. This research was conducted to enhance our understanding of the lily-type lectin gene in a fish for which the molecular features and function are not widely known.

2. Materials and methods

2.1. Cloning and sequencing rock bream lily-type lectin (RbLTL) cDNA

The full-length cDNA of the rock bream lily-type lectin (RbLTL) gene was obtained through expressed sequence tag (EST) analysis of a lipopolysaccharide (LPS) stimulated rock brema liver cDNA library (Kim et al., 2010).

The similarity analysis of the protein sequence was conducted using the BLAST program from the US National Center for Biotechnology Information (NCBI http://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple alignments of the RbLTL sequences were performed with the Genetyx (ver. 8.0) multiple sequence alignment program.

A phylogenetic tree was created using the MEGA4 software according to the neighbor-joining method based on the RbLTL sequences and known lily-type lectin family and mannose-binding lectin-like protein sequences with 2000 bootstrap replicates. The following lily-type lectin sequences with their GenBank accession numbers were used for the comparison: *Epinephelus coioides* AEG78370, *Leiognathus nuchalis* BAE79275, *Larimichthys crocea* ADN97105, and *Platycephalus indicus* BAE79274. The primary structure of the deduced RbLTL amino acid sequence was analysed with ProtParam (http://cn.expasy.org/tolls/protparm). The position was analysed with the Simple Modular Architecture Tool (http://smart.embl-deidelberg.de/).

2.2. Preparation of tissues, PBLs, egg, bacterial and viral infection in gills

Tissue-specific gene expression was analysed to evaluate the expression of RbLTL in peripheral blood leukocytes (PBLs), head kidney, trunk kidney, spleen, liver, intestine, gill and muscle. All of these tissues were isolated from three healthy rock bream weighing ~200 g according to the methods reported in our previous study (Kim et al., 2011). The PBLs was separated by density-gradient centrifugation using Percoll (Sigma-Aldrich) as described previously (Park et al., 2003).

For the bacterial challenge experiment, sub-lethal dose of *Streptococcus iniae* (1.5×10^5 cells/fish) and *Edwardsiella tarda* (1.5×10^5 cells/fish) were suspended in phosphate-buffered saline (PBS) buffer for infection by intra-peritoneal injection. For viral infection, red seabream iridovirus (RSIV) was isolated from rock bream farmed in the Republic of Korea and propagated and titrated as described previously (Caipang et al., 2003). Viral challenges were conducted at a dose of 1.1×10^4 copies/fish administered by intraperitoneal injection. The injected fish were kept in seawater at 23 ± 0.3 °C following each experimental challenge. PBS-injected rock bream (100 μL/fish) were used as controls. Gills were obtained from five fish 1, 3, 6, 12, 24, 36 and 48 h post-injection (pi) and frozen at −80 °C for RNA extraction.

For the time-series expression analysis of rock bream eggs, we sampled eggs at different times (1, 3, 6, 12, 24 and 36 h after fertilization). Eggs were also collected from an unfertilized control group.

2.3. Gene expression analysis of RbLTL in rock bream

The total RNA from each sample was extracted TRIzol® reagent (Invitrogen, Carlsbad, CA, USA), and the first-strand cDNA synthesis was performed using a first-strand cDNA synthesis kit (TaKaRa, Kyoto, Japan) according to the manufacturer's instructions.

The RbLTL mRNA expression levels were analysed by quantitative real-time PCR (qRT-PCR) with gene-specific primers (forward: 5′-GATGATGCTAACTTCGTCAT-3′, reverse: 5′-CGTCATCAGTCAGTTGAAGAC-3′) on a Thermal Cycler DICE Real-Time System (TaKaRa Bio, Inc.) using SYBR™ Green Master Mix (Takara, Kyoto, Japan). RbLTL expression was analysed using the comparative Ct ($2^{-\Delta\Delta CT}$) method with β-actin as a control (Kim et al., 2011). β-actin was amplified as a control using β-actin forward (5′- GGACACGGAAAGGATTGACA-3′) and β-actin reverse (5′- CGGAATTAACCAGACAAATC-3′) primers according to the methods reported in our previous study (Park et al., 2012). The data from each group were tested with a one-way analysis of variance (ANOVA) and the mean comparisons were performed using the Tukey multiple range test with SPSS version 17 software (SPSS, Chicago, IL, USA). All samples were analysed in triplicate, and the results are reported as the means ± the standard deviations (SDs).

3. Results and discussion

3.1. cDNA cloning and amino acid sequences of RbLTL

The rock bream *Oplegnathus fasciatus* in the family Oplegnathidae is one of the most economically important fish resource in Korea. This fish inhabits the Pacific Ocean, including southern parts of the Korean Peninsula, Japan, Taiwan, and Hawaii (Oh et al., 2007). This fish is infrequently compared with other commercially important fish in Korea. Red sea bream iridovirus (RSIV) disease is the major cause of rock bream mass mortality in Korea (Do et al., 2005). This study analysed the molecular features of a lily-type lectin in terms of its involvement with the immune functions of rock bream, which is an economically important fish for the Korean aquaculture industry.

In this study, the RbLTL cDNA contained several important signatures of the lily-type lectin family were identified in RbLTL. Database searches with the deduced RbLTL amino acid sequence revealed that RbLTL was 80% similar to the lily-type lectin from *Epinephelus coioides*. RbLTL contains a B-lectin domain or D-mannose binding sites similar to CRD (Fig. 1A), as follows: *Epinephelus coioides* AEG78370, *Leiognathus nuchalis* BAE79275, *Larimichthys crocea* ADN97105, and *Platycephalus indicus* BAE79274. The D-mannose binding sites (QxDxNxVxY) are likely to be involved in the specific binding of D-mannose sugar (Arasu et al., 2013). This result was showed that the D-mannose site are well-maintained, however, its exact functions has not well-known in teleosts. Accordingly, further investigation concerning the function of this