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# Anti-viral activity of galectin-1 from flounder *Paralichthys olivaceus*

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ABSTRACT

Galectins are a family of $Ca^{2+}$-independent soluble lectins characterized by their affinity to β-galactosides. Mammalian galectins have been shown to play a defense role against certain bacteria, fungi and viruses. However, the immunological functions of galectins in fish is poorly characterized. Here we demonstrated that the expression of galectin-1 gene from the flounder *Paralichthys olivaceus* was decreased in the initial 8 h after challenge with poly I:C, then increased markedly from 24 h onwards, and the recombinant galectin-1 was able to neutralize the lymphocystis disease virus (LCDV), inhibiting the formation of cytopathic effects. In addition, the recombinant galectin had a potential anti-inflammatory activity against infection by LCDV, and was able to restrain the overexpression of the anti-viral protein gene *mx* against virus infection. These results indicate that flounder galectin-1 has an anti-viral activity, capable of reducing LCDV pathogenicity.



## 1. Introduction

The innate immune system is the first line of defense protecting the host from invasion by pathogenic microbes, including viruses in vertebrates and the only defense arsenal against microbes in invertebrates that are devoid of an adaptive immune system. Many components of innate immune system are evolutionarily conserved from sea anemone to humans [1]. In both vertebrates and invertebrates, the response to invading pathogens is initiated by the detection of pathogen-associated molecular patterns (PAMPs), present in microbes but absent in the host, by host pattern recognition receptors (PRRs) [2,3].

Lectins are well-known PRRs that specifically bind to the carbohydrate molecules on the surface of pathogens, and help in their rapid clearance by enhancing opsonization and phagocytosis and also by increasing the oxidative burst activities [3–8]. Animal lectins are classified into distinct families characterized by unique sequence motifs and structural folds: galectins, C-type lectins (including selectins, collectins and hyalectins), F-type lectins, I-type lectins, P-type lectins (Man-6-P-lectins), pentraxins, ficolins and cytokine lectins [9] (see also www.imperial.ac.uk/research/animallectins). Although lectins from the various families differ vastly in their domain architecture, they all have at least one carbohydrate-recognition domain (CRD), conferring the protein its carbohydrate-binding capacity [10].

Galectins, formerly known as S-type lectin, are a family of $Ca^{2+}$-independent soluble lectins characterized by their affinity to β-galactosides. Based on structural features, galectins have been classified into proto-, chimera- and tandem-repeat types [11]. Prototype galectins possess one CRD per unit, and usually form homodimers of noncovalently linked subunits. In contrast, chimera-type galectins are monomeric with a C-terminal CRD, joined to an N-terminal short region of yet unknown functional properties. Tandem-repeat type galectins are also monomeric, with two homologous CRDs joined by a short linker region. Recently, a novel tandem-repeat type galectin with four CRDs has been identified in the oyster *Cassotrea virginica* [12]. To date, 15 galectins have been identified in mammalian species [8]. In mammals, the roles of galectins in innate host defense against certain bacteria, fungi and viruses have been well documented. For example, galectin-1 was shown to have antiinflammatory activity by inhibiting leukocyte infiltration, migration and recruitment, and galectin-3 to have proinflammatory activity by enhancing macrophage survival and recruitment [13]. Moreover, galectin-1 was shown to bind to influenza virus and ameliorates pathogenesis [14]. Galectins have been isolated from several fish species including flounder, medaka, Atlantic salmon, rainbow trout and pufferfish [15]. Recently, galectin-1 from sea bass was demonstrated to have potential anti-inflammatory activity [16]. However, the immunological functions

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of galectins in fish remain poorly characterized. The aims of this were therefore to clone and identify the galectin-1 gene from flounder *Paralichthys olivaceus*, named *fglec-1*, and to examine the anti-viral activity and immunomodulatory activity of the recombinant fGLec-1.

## 2. Materials and methods

### 2.1. Cell line and virus

The continuous cell line FG-9307 used was established from the gills of *P. olivaceus* in our laboratory. The cells were cultured in minimal essential medium (MEM, HyClone) supplemented with 10% bovine calf serum (BCS, HyClone), 100 IU/ml penicillin and 100 mg/ml streptomycin, in plastic cell culture flasks (Corning) at 22 °C as described [17]. Lymphocystis disease virus (LCDV) was a gift of Dr. Guobin Hu (Ocean University of China). The viral titers were measured by a 50% tissue culture infective dose ($TCID_{50}$) assay according to the method [18].

### 2.2. RNA extraction and cDNA synthesis

The head kidney was dissected out of the flounder *P. olivaceus*, grounded in RNAiso plus (TaKaRa) and kept at −70 °C until use. Total RNAs were extracted from the frozen sample according to the manufacturer's instructions. The cDNA was synthesized with reverse transcription system (Promega) using oligo (dT) primer after digestion with recombinant DNase I (RNase free) (TaKaRa) to eliminate the genomic contamination. The reaction was carried out at 42 °C for 50 min and inactivated at 75 °C for 15 min. The cDNAs synthesized were stored at −20 °C until use.

### 2.3. Cloning and sequencing of galectin-1 cDNA

A pair of specific primers P1 and P2 (Table 1) was designed according to the sequence of flounder galectin-1 (GenBank accession number: AF220550.1; http://www.ncbi.nlm.nih.gov/) using Primer Premier 5.0 program. The PCR amplification reaction was carried out at 94 °C for 5 min, followed by 32 cycles at 94 °C for 30 s, 57 °C for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 7 min. The amplification products were gel-purified using DNA gel extraction kit (AXYGEN), cloned into the pGEM-T vector (Invitrogen), and transformed into Trans5α *Escherichia coli* (TransGen). The positive clones were selected and sequenced to verify for authenticity.

**Table 1**
Sequences of the primers used in this study.

| Primer | Sequence (5′–3′) | Sequence information |
|---|---|---|
| P1 (sense) | CACTGTCATTTCTTCCCAGCCGAG | Flounder galectin |
| P2 (antisense) | CTCCTCATCCCCCAACCAACTGT | clonging primer |
| P3 (sense) | GAATATTGGCCCCACTGACCAGGAC | Real-time PCR primer |
| P4 (antisense) | TGGAAAGGAAAGCCTCCCTCACG | (galectin) |
| P5 (sense) | CCCATCTACGAGGGCTACGC | Real-time PCR primer |
| P6 (antisense) | TCTCGGCTGTGGTGGTGAAG | (β-actin) |
| P7 (sense) | GGAATTCCATATGATGATGAAAAAC ATGATGATAAAGA | Recombinant primer |
| P8 (antisense) | CCGCTCGAGTTTACTTGATCTCAAA GCTTCTGATG | |
| P9 (sense) | GCAGCAACCGCAAAGTTCTTCTCAA | Real-time PCR primer |
| P10 (antisense) | GACACGCTCCAGATGCAGGGTT | (IL-1β) |
| P11 (sense) | CCGCACGGGACACAACTTTAACATG | Real-time PCR primer |
| P12 (antisense) | TGGTTCTCCAGCTTGTTTCATCTGC | (Mx) |
| P13 (sense) | AGTCGTCCACCGACTGGATGTGTAA | Real-time PCR primer |
| P14 (antisense) | GGTTCTGTTTTCTCAGCGCAGTCCT | (TNF-α) |

### 2.4. Sequence and phylogenetic analyses

The protein domain was analyzed using the SMART program (http://smart.embl-heidelberg.de/). Signal prediction was conducted using SignalP 3.0 Server (http://www.cbs.dtu.dk/services/SignalP/). The molecular masses (MW) and isoelectronic points (PI) of the mature peptide was determined using ProtParam (http://www.expasy.ch/tools/protparam.html). The three-dimensional structure prediction was performed by SWISS-MODEL online software at the Expert Protein Analysis System (http://www.expasy.org/). Homology searches in the GenBank database were carried out by BLAST server (http://www.ncbi.nlm.nih.gov/BLAST/) and multiple alignments of the protein sequences and homologies among CRD domains were generated using the Clustal W program within the MegAlign of the DNASTAR software package (version 5.0). Phylogenetic trees were constructed by MEGA (version 4.1) using p-distance based on the neighbor-joining method. The reliability of each node was estimated by bootstrapping with 1000 replications.

### 2.5. Semi-quantitative real time polymerase chain reaction (qRT-PCR)

Juvenile flounder *P. olivaceus* with weight of 55 ± 5 g ($n = 60$) were purchased from a commercial fish farm (Weihai, Shandong province, China). The fishes were acclimatized in tanks with continuously aerated seawater at room temperature for a week and then divided equally into two groups, experimental and control groups. The fishes in the experimental group were intraperitoneally (i.p.) injected with poly I:C (5 mg/ml, 100 μl per fish), and the control fishes were injected with the same volume of phosphate buffered saline (PBS). Three fishes were sampled from each group at 2, 4, 8, 12, 24, 48 and 72 h post injection and the head kidneys were dissected out of each fish. Total RNAs were prepared with RNAiso plus (TaKaRa) from the head kidneys, and cDNAs were synthesized with reverse transcription system using oligo (dT) primer as above used as template. The primers P3 and P4 specific for *fglec-1* and P5 and P6 specific for *β-actin* were designed using Primer Premier 5.0 program (Table 1). qRT-PCR was performed on ABI 7500 Real-time PCR system (Applied Biosystems, USA) as described [19]. The β-actin gene was chosen as the reference for internal standardization.

### 2.6. Construction of expression vector

The full length of *fglec-1* was amplified by PCR as above using the upstream primer P7 and the downstream primer P8 (Table 1). The PCR products were digested with NdeI and XhoI and subcloned into the plasmid expression vector pET-28a (Novagen) previously cut with the same restriction enzymes. The identity of inserts were verified by sequencing and the plasmid was designated *pET-28a/fglec-1*.

### 2.7. Expression and purification of recombinant fGLec-1

The plasmid *pET-28a/fglec-1* was transformed into *E. coil* transetta (DE3), and the cells were cultured overnight in LB broth containing kanamycin (100 μg/ml). The induction of protein synthesis was performed as described [20] with a slight modification. The culture was diluted 1:50 with LB broth and subjected to further incubation at 37 °C until $OD_{600}$ reached about 1.0. The expression of fGLec-1 was induced by addition of isopropyl β-D-thiogalactoside (IPTG) to the culture at a final concentration of 0.1 mM. After further incubation at 37 °C for 4 h, the bacterial cells were harvested by centrifugation at 5000 × g at 4 °C for 10 min, resuspended in 40 ml buffer A (pH8.0) consisting of 50 mM Tris and