Fish & Shellfish Immunology 44 (2015) 584–591

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

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

Full length article

A galectin from shrimp *Litopenaeus vannamei* is involved in immune recognition and bacteria phagocytosis

Fujun Hou [a], Yongjie Liu [a], Shulin He [a], Xianzong Wang [a], Aitao Mao [b], Zhigang Liu [b], Chengbo Sun [b], Xiaolin Liu [a, *]

[a] College of Animal Science and Technology, Northwest A&F University, Shaanxi Key Laboratory of Molecular Biology for Agriculture, Yangling 712100, China
[b] Fisheries College, Guangdong Ocean University, Guangdong 524088, China

ARTICLE INFO

Article history:
Received 12 September 2014
Received in revised form
10 March 2015
Accepted 13 March 2015
Available online 26 March 2015

Keywords:
Pattern recognition receptor
Galectin
Litopenaeus vannamei
Bacteria

ABSTRACT

Galectins are conserved family members with β-galactosides affinity that play multiple functions in embryogenesis, development and regulation of innate and adaptive immunity. However, little functional studies were reported in crustaceans. Here, a shrimp *Litopenaeus vannamei galectin* (*LvGal*) cDNA was identified with an open reading frame of 1017 bp, which encodes a putative protein of 338 amino acids. A carbohydrate recognition domain (CRD) and several amino acids residues involved in dimerization were found in *Lv*Gal. *LvGal* mRNA was mainly expressed in gills and hemocytes and upregulated post *Vibrio anguillarum* challenge. Recombinant *Lv*Gal (r*Lv*Gal) was expressed in *Escherichia coli* BL21 (DE3) and the purified r*Lv*Gal could strongly bind G^- bacteria *V. anguillarum* and G^+ bacteria *Micrococcus lysodeikticus*. Besides, r*Lv*Gal exhibited strong activity to agglutinate *V. anguillarum* and weak activity to agglutinate *M. lysodeikticus* but no obvious antibacterial activity was found with selected bacteria. In addition, in vivo experiments showed r*Lv*Gal could promote phagocytosis of bacteria by hemocytes. Thus, through these collective data we predicted *Lv*Gal is involved in immune recognition and functions as a potential pattern recognition receptor.

1. Introduction

The massive loss of shrimp production caused by bacteria or virus infection compels us to fully understand the shrimp innate immunity. Like other invertebrates, shrimps depend on cellular defenses and humoral defenses to protect themselves from infection. Briefly, the pattern recognition receptors (PRRs) of shrimps recognize pathogen associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS) from Gram-negative bacteria and peptidoglycan (PGN) from Gram-positive bacteria. And the activated PRRs directly or indirectly trigger a series of shrimp cellular or humoral responses such as the activated prophenoloxidase system, hemolymph clotting mechanism, hemocytes phagocytosis and the release of NF-κB dependent antimicrobial peptides [1].

Galectins are a large conserved family members existing in mammals, insects and even fungi. Proteins with two characteristics are defined as galectins: an about 130 amino acids carbohydrate recognition domain (CRD) and the affinity for β-galactosides [2]. Based on their molecular structures, galectins are classified into proto-type, chimera-type and tandem-repeat-type. The proto-type galectins contain one CRD and chimera-type galectins contain an N-terminal domain rich in proline and glycine besides a C-terminal CRD. While tandem-repeat-type galectins has two CRDs linked by a peptide [3]. However, novel tandem-repeat-type galectins with four CRDs are found in some species as bay scallop *Argopectens irradians* [4]. Previous reports showed that although galectins did not contain a classical secretion signal peptide, they were found not only in cytoplasm and nucleus but also in the extracellular space [5,6]. Up to 15 distinct galectins have been found in mammals and demonstrated to play multiple functions in embryogenesis, development and regulation of innate and adaptive immunity [7–9]. In terms of mammalian innate immunity, galectins played vital roles. For example, galectin 1 performs anti-inflammatory activities by blocking or attenuating signaling events that lead to leukocyte infiltration, migration and recruitment [10,11]. However, galectin 3 shows pro-inflammatory activity through anti-apoptotic activity for macrophages and enhancing their interactions with basal lamina glycans, such as laminin and fibronectin [12]. Galectin 9

* Corresponding author. Tel.: +86 029 87054333; fax: +86 029 87092164.
E-mail address: liuxiaolin@nwsuaf.edu.cn (X. Liu).

http://dx.doi.org/10.1016/j.fsi.2015.03.017
1050-4648/

induces chemotaxis, activation, oxidative activity and degranulation of eosinophils [13]. In contrast with their roles in innate immunity, galectins also function in mammalian adaptive immune response by regulating immune cell development and homeostasis [14,15]. Galectin-1 can regulate proliferation and apoptosis of T cell by binding and collecting of lactosamine-rich cell surface glycolconjugates into segregated membrane microdomains [16]. Depending on the different developmental stage and activation level, galectin 1 can have pro- or anti-apoptotic roles on T cells [17]. Galectin 3 could prevent apoptosis of T cells and promote adhesion of thymocytes to thymic epithelium [12,18]. Besides, recent findings show that galectins could bind glycans on the surface of pathogenic microbes, indicating their role as pattern recognition receptors (PRRs) [19–21]. However, few studies about galectins were reported in invertebrates compared with relatively detailed studies in mammals. *Drosophila melanogaster* galectin was identified with two CRDs connected by a peptide link. In contrast, the putative *Anopheles* galectin is predicted to contain only one CRD [22]. The tissue distribution of Drosophila galectin in somatic and visceral musculature, central nervous system, hemocytes and circulating phagocytic cells suggest its role in embryogenesis, host defense and immune response [23]. While, the Anopheles galectin may agglutinate and opsonize bacteria and contribute to limiting bacterial growth in the midgut following blood-feeding [24]. The temporal expression of pearl oyster *Pinctada fucata* galectin mRNA significantly increased post *Vibrio alginolyticus* infection [25]. The recombinant bay *scallop A. irradians* galectin could agglutinate bacteria *Escherichia coli*, *Vibrio anguillarum*, *Vibrio fluvialis*, *Edwardsiella tarda* and *Micrococcus luteus*. Besides, it could bind to hemocytes and enhance its encapsulation of agarose beads [26]. As for crustaceans, only one report was found from the Kuruma Shrimp (*Marsupenaeus japonicus*) which functions as an opsonin and promotes bacterial clearance from hemocytes [27]. Hence, considering different galectin homologues might have different roles as well as the increasingly vital roles of galectins in mammalian innate immunity, we take this interest to do this research and want to show whether galectin in shrimp *Litopenaeus vannamei* also has similar immune functions.

2. Materials and methods

2.1. cDNA cloning and bioinformatics analysis

Shrimps *L. vannamei* (8–10 g each body weight) were presented from Hengxing shrimp base (Guangdong, China). And they were cultured in the aquaculture lab of Fisheries College, Guangdong Ocean University under comfortable temperature and circulatory sea water. After temporary rearing for 5 days, gills of three shrimps were sampled and used for total RNA extraction. Methods for total RNA extraction and cDNA synthesis were based on manufacturer's instructions and had been described in our previous study [28]. The RT Primer Mix of Takara including oligo dT primers and random 6 hexamers was used for reverse transcription. Primers used for cDNA cloning of shrimp *L. vannamei* galectin gene (*LvGal*) were designed based on the reported *M. japonicus* galectin cDNA (GenBank No. JQ804931.1) (Table 1). PCR conditions were as follows: 94 °C for 4 min; 30 cycles of 94 °C for 30 s, 52 °C for 30 s and 72 °C for 1 min; 72 °C for 5 min. The PCR products were purified with Universal DNA Purification Kit (TIANGEN, China) and cloned into pUC-T vector using pUC-T Ligasing Kit (CWBIO, China). The positive clones were sequenced in Genescript Company (Nanjing, China). Then we use the obtained sequence to online blast Transcriptome Shotgun Assembly (TSA) database of Pacific white shrimp (taxid:6689) in NCBI, and the almost same sequence was got with GenBank: JP423263.1. According to this TSA sequence, we designed a reverse primer *Lv*Gal R1 (Table 1) for 5′ RACE following the protocol of SMARTer™ RACE cDNA Amplification Kit User Manual (Clontech). After PCR, gel-purification, sequencing, *Lv*Gal cDNA with partial 5′ and 3′ sequence was got.

Table 1
PCR primers used in this study.

Target gene name	Primer name	Sequence (5′–3′)
cDNA cloning		
LvGal	*Lv*Gal-F	atgtcagcaccagtgtacaacc
	*Lv*Gal-R	tcactcctcttcactggagccagaa
	*Lv*Gal-R1	tgcatacactgaagcatattactga
Protein expression[a]		
LvGal	r*Lv*Gal-F	cgggatccatgtcagcaccagtgtacaacc
	r*Lv*Gal-R	Ccgctcgagtctcctcttcactggagcca
qPCR analysis		
LvGal	qGal-F	ccaatgtatccaaatcagccctat
	qGal-R	tgctgccatacccaataatcct
18s rRNA	18s-F	aacgctcgtagtttgacttctgc
	18s-R	cacgaccattcgggctgta

[a] BamHI and XhoI sites are underlined.

The basic properties of *Lv*Gal amino acids were analyzed by the Expert Protein Analysis System http://www.expasy.org/. Signal peptides of *Lv*Gal was explored at http://www.cbs.dtu.dk/services/SignalP/. Conserved domains were predicted at http://smart.embl-heidelberg.de/. SWISS-MODEL prediction algorithm (http://swissmodel.expasy.org/) was used to simulate the 3-D model of *Lv*Gal. Multiple sequence alignment was analyzed with ClustalW of MegAlign. Two phylogenetic trees were built with Mega software, one including 15 kinds of mammalian galectins with LvGal while the other including vertebrates and invertebrates galectins.

2.2. Tissue distribution of LvGal mRNA

Tissue distribution of LvGal was carried out by sampling three adult shrimps (mean body weight 40–45 g) which were from East Sea Island Base of Guangdong Ocean University. Total RNA were extracted from eight tissues including hemocytes, gills, heart, hepatopancreas, stomach, intestine, eyestalk and muscle. Hemolymph was draw from the ventral sinus using a 1 ml sterile syringe preloaded with 400 μl of anticoagulant (0.1 M glucose, 30 mM citrate, 26 mM citric acid, 0.14 M NaCl, 10 mM EDTA) followed by centrifugation at 800 g at 4 °C to isolate hemocytes. Semi-quantitative PCR was used to detect tissue distribution of *LvGal*. Specific primers were designed for *LvGal* and *L. vannamei 18s rRNA* (*18s*, GenBank No. AF186250) was used as internal control (Table 1). To confirm exponential stage of PCR, we have carried out preliminary experiment using gradient cycles with 17, 19, 21, 23, 25 cycles for *18s rRNA* and 26, 28, 30, 32, 35 cycles for *LvGal*. After that, 6 μl PCR products were run on 1% agarose gel and detected using Gel Imaging System (Bio-rad, USA). By comparison of brightness and gray values of different cycles, the values did not increase obviously after 21 cycles for *18s rRNA* and 32 cycles for *LvGal*, so at last we chose 19 cycles for *18s rRNA* and 30 cycles for *LvGal* to complete semi-quantitative PCR. PCR conditions were as follows: 94 °C for 4 min; 30 cycles for *LvGal* or 19 cycles for *18s rRNA* of 94 °C for 30 s, 57 °C for 20 s and 72 °C for 20 s; 72 °C for 5 min.

2.3. Responses of LvGal post bacteria challenge

Gram-negative bacteria *Vibrio anguillarum* (*V. anguillarum*) and Gram-positive bacteria *Micrococcus lysodeikticus* (*M. lysodeikticus*) were gifted by professor Fuhua Li. The method for bacteria preparation and quantification was same as our previous study [28]. Healthy shrimps (8–10 g each body weight) with a total of 90 were