Developmental and Comparative Immunology 46 (2014) 231–240

Contents lists available at ScienceDirect

Developmental and Comparative Immunology

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

Identification of a C-type lectin with antiviral and antibacterial activity from pacific white shrimp *Litopenaeus vannamei*

Ming Li [a,c,1], Chaozheng Li [a,1], Chunxia Ma [d], Haoyang Li [a], Hongliang Zuo [a], Shaoping Weng [a], Xiaohan Chen [c], Digang Zeng [c], Jianguo He [a,b,*], Xiaopeng Xu [a,*]

[a] MOE Key Laboratory of Aquatic Product Safety/State Key Laboratory for Biocontrol, School of Life Sciences, Sun Yat-sen University, Guangzhou, PR China
[b] School of Marine Sciences, Sun Yat-sen University, Guangzhou, PR China
[c] Guangxi Key Laboratory of Aquatic Genetic Breeding and Healthy Aquaculture, Guangxi Institute of Fisheries, Nanning, PR China
[d] Guangxi Veterinary Research Institute, Guangxi University, Nanning, PR China

ARTICLE INFO

Article history:
Received 9 February 2014
Revised 20 April 2014
Accepted 22 April 2014
Available online 1 May 2014

Keywords:
C-type lectin
Litopenaeus vannamei
NF-κB signaling pathway
Innate immunity
Agglutination
WSSV

ABSTRACT

C-type lectins (CTLs) play crucial roles in innate immune responses in invertebrates by recognizing and eliminating microinvaders. In this study, a CTL from pacific white shrimp *Litopenaeus vannamei* (LvCTL3) was identified. LvCTL3 contains a single C-type lectin-like domain (CTLD), which shows similarities to those of other shrimp CTLs and has a mutated 'EPD' motif in Ca^{2+}-binding site 2. LvCTL3 mRNA can be detected in all tested tissues and expression of LvCTL3 in gills was up-regulated after Lipopolysaccharides, poly (I:C), *Vibrio parahaemolyticus* and white spot syndrome virus (WSSV) challenges, suggesting activation responses of LvCTL3 to bacterial, virus and immune stimulant challenges. The 5′flanking regulatory region of LvCTL3 was cloned and we identified a NF-κB binding motif in the LvCTL3 promoter region. Dual-luciferase reporter assays indicated that over-expression of *L. vannamei* dorsal can dramatically up regulate the promoter activity of LvCTL3, suggesting that LvCTL3 expression could be regulated through NF-κB signaling pathway. As far as we know, this is the first report on signaling pathway involve in shrimp CTLs expression. The recombinant LvCTL3 protein was expressed in *Escherichia coli* and purified by Ni-affinity chromatography. The purified LvCTL3 can agglutinate Gram-negative microbe *Vibrio alginolyticus* and *V. parahaemolyticus* and Gram-positive bacteria *Bacillus subtilis* in the presence of calcium ions, but cannot agglutinate Gram-positive bacteria *Streptococcus agalactiae*. The agglutination activity of LvCTL3 was abolished when Ca^{2+} was chelated with EDTA, suggesting the function of LvCTL3 is Ca^{2+}-dependent. *In vivo* challenge experiments showed that the recombinant LvCTL3 protein can significantly reduce the mortalities of *V. parahemolyticus* and WSSV infection, indicating LvCTL3 might play significant roles in shrimp innate immunity defense against bacterial and viral infection.

1. Introduction

Innate immunity is the main defense for invertebrates against threats from pathogens (Akira, 2009; Akira et al., 2006). Similar to those in vertebrates, innate immune responses against non-self invaders in invertebrates are initiated and primed by immune recognition (Akira, 2009; Akira et al., 2006). A range of germline-encoded molecules termed pattern recognition receptors (PRRs) that can recognize evolutionarily conserved pathogen-associated molecular patterns (PAMPs) on the surface of invading microorganisms play essential roles during the immune recognition process (Akira, 2009; Akira et al., 2006). In invertebrates, a number of PRRs have been identified, including Toll-like receptors (TLRs), scavenger receptors (SCRs), thioester-containing proteins (TEPs), peptidoglycan recognition proteins (PGRPs), Gram-negative binding proteins (GNBPs), fibrinogen-related proteins (FREPs), and lectins (Medzhitov, 2007; Iwanaga and Lee, 2005; Kim and Kim, 2005; Beutler, 2004). They can recognize many different PAMPs, such as lipopolysaccharide (LPS) from Gram-negative bacteria, peptidoglycan and lipoteichoic acid (LTA) from Gram-positive bacteria, mannans from fungi, and double stranded RNAs or glycoproteins from viruses, and then trigger a series of antimicrobial responses (Medzhitov, 2007; Iwanaga and Lee, 2005; Kim and Kim, 2005; Beutler, 2004).

* Corresponding authors. Address: School of Life Sciences, School of Marine Sciences, Sun Yat-sen (Zhongshan) University, Guangzhou 510275, PR China. Tel.: +86 20 39332988; fax: +86 20 84113229 (J. He). Address: School of Life Sciences, Sun Yat-sen (Zhongshan) University, Guangzhou 510275, PR China. Tel.: +86 20 84113793; fax: +86 20 84113229 (X. Xu).
E-mail addresses: lsshjg@mail.sysu.edu.cn (J. He), xuxpeng@mail.sysu.edu.cn (X. Xu).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.dci.2014.04.014
0145-305X/

C-type lectins (CTLs), a large super-family of lectins characterized by containing at least one C-type lectin-like domains (CTLDs), are present in almost all living organisms (Robinson et al., 2006; Ofek et al., 2000). CTLs bind specific carbohydrates on the surfaces of pathogens through CTLD in the presence of calcium ions (Robinson et al., 2006; Ofek et al., 2000; Vasta et al., 1999; Drickamer, 1999). CTLD, the carbohydrate recognition domain (CRD) of CTLs commonly 110–130 residues in length, has a characteristic double-loop stabilized by two conserved disulfide bonds, and contains four Ca^{2+}-binding sites involved in structure maintenance and carbohydrates binding (Robinson et al., 2006; Ofek et al., 2000; Vasta et al., 1999; Drickamer, 1999). The Ca^{2+}-binding site 2 of most CTLs has a conserved 'EPN' or 'QPD' motif that is important for mannose or galactose binding, respectively (Robinson et al., 2006; Ofek et al., 2000; Vasta et al., 1999; Drickamer, 1999).

A number of CTLs have been identified in various invertebrates, such as nematodes, shellfishes, insects and crustaceans (Wang et al., 2013; Boman and Hultmark, 1987; Wang and Wang, 2013; Schulenburg et al., 2008). Invertebrate CTLs work to eliminate invading pathogens through directly agglutinating or killing microorganisms, stimulating melanization or hemocyte encapsulation, and promoting signal transduction to evoke humoral immune responses. CTLs from shrimps are representative ones among invertebrates CTLs (Ofek et al., 2000; Wang and Wang, 2013). They usually contain one or two CTLDs and their expressions can be activated by pathogen infection or stimulant challenge (Vasta et al., 1999; Schulenburg et al., 2008). In recent years, a growing number of CTLs have been reported in pacific white shrimp *Litopenaeus vannamei*, Chinese white shrimp *Fenneropenaeus chinensis*, tiger prawn *Penaeus monodon*, kuruma prawn *Marsupenaeus japonicus*, and banana shrimp *Fenneropenaeus merguiensis* (Schulenburg et al., 2008; Zhang et al., 2009; Zhao et al., 2009; Junkunlo et al., 2012; Lai et al., 2013; Liu et al., 2007; Rattanaporn and Utarabhand, 2011; Rittidach et al., 2007). Shrimps are susceptible to a wide range of pathogens that bring great damages to farming industry (Mine and Boopathy, 2011). Exploring novel CTLs in shrimps is important for understanding the crustacean innate immune system and can help develop tools and strategies for protecting shrimps against pathogen infections. In this study, a CTL from *L. vannamei*, termed LvCTL3 distinguished from previously identified LvCTL1 and LvCTL2 (Wei et al., 2012), with agglutination activity was identified and its anti-bacterial and anti-viral activities were examined *in vivo*. Moreover, dual-luciferase reporter assays showed that expression of LvCTL3 was promoted by LvDorsal, suggesting that LvCTL3 expression could be regulated through NF-κB signaling pathway.

2. Materials and methods

2.1. Cloning of the shrimp LvCTL3 cDNA

A partial cDNA sequence that is homologous to shrimp CTLs was retrieved from the sequenced *L. vannamei* transcriptome data (Li et al., 2012). The full length cDNA sequence was then obtained using the rapid amplification of cDNA ends (RACE) method. Briefly, total RNA was extracted from *L. vannamei* gills with the RNeasy Plus Mini Kit (QIAGEN, USA). The cDNA template for RACE-PCR was prepared using the SMARTer™ RACE cDNA Amplification kit (Clontech, Japan). 5′-RACE-PCR amplification was performed with Universal Primer A Mix (UPM) and LvCTL3 specific reverse primer 5RACE1. Nested PCR was subsequently performed with Nested Universal Primer A (NUP) and LvCTL3–5RACE2 using the first-round PCR product as template. 3′-RACE-PCR was performed using UPM together with an LvCTL3-specific forward primer 3RACE1, and the nested PCR was subsequently performed with NUP and LvCTL3–3RACE2. The second PCR products were cloned into pMD-20T vector (TaKaRa, Japan) and 12 positive clones were selected and sequenced (ABI PRISM, Applied Biosystems, USA). 5′-RACE is a technique used in molecular biology to obtain the 5′ UTR of a transcript and identify the transcription starting site (TSS) of promoter elements (Olivarius et al., 2009; Scotto-Lavino et al., 2006). TSS of LvCTL3 is determined according to the 5′-RACE PCR amplification.

2.2. Genome walking

Genome walking is a technology to identify the flanking genomic segments (e.g. promoter regions) adjacent to a known sequence (Leoni et al., 2008). The 5′ flanking regulatory region of LvCTL3 was isolated by Genome walking method. The *L. vannamei* genome DNA was prepared according to the protocol as previously described (Koyama et al., 2010). Genome walking libraries were constructed by GenomeWalker™ Universal Kit (Clontech, Japan) according to the manufacturer's recommendations. The primer pairs AP1/5′GW-LvCTL3–1 and AP2/5′GW-LvCTL3–2 were used to perform the first and second rounds of genome walking PCR amplification, respectively. The amplicon was cloned to pMD-20T vector (TaKaRa, Japan) and sequenced.

2.3. Bioinformatics analysis

Protein sequences of C-type lectin homologs from other species were retrieved from the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/) databases using the BLAST program (basic local alignment search tool). The CTLD domain of LvCTL3 were modeled by homology using 3D-JIGSAW webserver tool (http://bmm.cancerresearchuk.org/~3djigsaw/) with the default settings. Sequence alignments between LvCTL3 and CTLs from other species were analyzed using clustal X v2.0 program (Aiyar, 2000) and were visualized by Espript3.0 (http://espript.ibcp.fr/ESPript/cgi-bin/ESPript.cgi). Phylogenetic trees were constructed based on the deduced amino acid sequences using MEGA 5.0 software, applying the amino acid substitution type and poisson model and bootstrapping procedure with a minimum of 1000 bootstraps (Tamura et al., 2011). Protein domains were predicted using the SMART program (http://smart.embl-heidelberg.de/). The 5′ flanking promoter sequences of LvCTL3 were analyzed for potential transcription factor binding sites with TRANSFAC® 6.0 program (Wingender, 2008) using high quality matrices and 0.85 as matrix and core similarity cut-off.

2.4. Real-time RT-PCR

Healthy specific-pathogen-free (SPF) shrimp *L. vannamei* with an average weight of 5 g were obtained from national SPF *L. vannamei* farm of Fangchenggang, Gunagxi province. For tissue expression analysis, shrimp tissues, including hepatopancreases, hemocytes, gills, eyestalks, muscles, scapes, epitheliums, pyloric ceca, intestines, and stomachs were sampled and pooled from 15 shrimps. For challenge experiments, shrimps were cultured in salt-water tanks at room temperature (27 °C) and divided into 4 experimental groups, in which *L. vannamei* was injected at the second abdominal segment with 2 μg/μl poly (I:C), 2 μg/μl LPS, 10^6 CFU (colony-forming unit) of *Vibrio parahaemolyticus*, and 10^6 copies newly extracted WSSV particles in 50 μl DEPC-treated water prepared PBS solution (pH 7.4), respectively, as well as a control group injected with 50 μl PBS (Ai et al., 2008). Gills of challenged shrimps were sampled at 0, 4, 8, 12, 24, 36, 48, 72 h post injection