Developmental and Comparative Immunology 38 (2012) 332–341

Contents lists available at SciVerse ScienceDirect

Developmental and Comparative Immunology

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

A novel short peptidoglycan recognition protein in amphioxus: Identification, expression and bioactivity

Feng Yao [a,b], Zhaohe Li [a], Yanjie Zhang [a], Shicui Zhang [a,*]

[a] Laboratory for Evolution & Development, Institute of Evolution & Marine Biodiversity and Department of Marine Biology, Ocean University of China, Qingdao 266003, China
[b] School of Life Science, Liaoning Normal University, Dalian 110069, China

ARTICLE INFO

Article history:
Received 20 May 2012
Revised 21 July 2012
Accepted 22 July 2012
Available online 7 August 2012

Keywords:
Amphioxus
Peptidoglycan recognition protein
Chitin-binding domain
Amidase
Microbicidal activity

ABSTRACT

Peptidoglycan recognition proteins (PGRPs) are widely distributed in invertebrates and vertebrates, and structure–activity relationship of insect and mammalian PGRPs has been well characterized, but functional and structural insights into PGRPs in other species are rather limited. Here we identified a novel short PGRP gene from the amphioxus *Branchiostoma japonicum*, named *pgrp-s*, which possesses a domain combination of ChtBD1 domain-PGRP domain, which is unique to all known PGRPs. Amphioxus *pgrp-s* was predominantly expressed in the hepatic caecum, hind-gut and muscle in a tissue-specific manner. Recombinant PGRP-S, rPGRP-S, and truncated protein with ChtBD1 domain deleted, rP86/250, both showed affinity to Dap-type PGN, Lys-type PGN and chitin. Consistently, they were also able to bind to *Escherichia coli*, *Staphylococcus aureus* and *Pichia pastoris*. Moreover, both rPGRP-S and rP86/250 had amidase enzymatic activity, capable of hydrolyzing Dap-type and Lys-type PGNs. Like vertebrate PGRPs, rPGRP-S was directly microbicidal, capable of killing *E. coli*, *S. aureus* and *P. pastoris*, whereas rP86/250 only inhibited the growth of *E. coli* and *S. aureus*, and its anti-*P. pastoris* activity was significantly reduced. It is clear that neither the binding of amphioxus PGRP-S nor its amidase enzymatic activity depend on the N-terminal ChtBD1 domain, but its antifungal activity does. Collectively, these data suggested that amphioxus PGRP-S may function as a multivalent pattern recognition receptor, capable of recognizing PGN and chitin, a microbicidal agent, capable of killing bacteria such as *E. coli* and *S. aureus* and fungus like *P. pastoris*, and probably a PGN scavenger, capable of hydrolyzing PGN.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

The innate immune system is the first line of defense protecting the host from invasion by microbes in vertebrates and the only defense arsenal against microbes in invertebrates that are devoid of an adaptive immune system. It arose in the early multicellular organisms and has remained an essential part of defense mechanisms in all metazoans (Irazoqui et al., 2010). Many components of innate immune system are evolutionarily conserved from sea anemone to humans (Hemmrich et al., 2007). Innate immunity is initiated by host's recognition of microbes via pattern recognition receptors (PRRs), such as Toll-like receptors and lectins, that bind conserved stereotypical molecular structures called pathogen-associated molecular patterns (PAMPs), present in microbes but absent in the host (see review by Janeway and Medzhitov, 2002; Hoffmann, 2003). Representative PAMPs include lipopolysaccharide (LPS), lipoteichoic acid (LTA), non methylated CpG sequences, and peptidoglycan (PGN).

PGN is an essential and specific cell wall component of virtually all bacteria and, as such, comprises an ideal target for recognition by PRRs. PGN is a polymer of β (1-4)-linked *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc). MurNAc is attached to short peptides, containing three to five alternating L and D amino acids. According to the residue at position 3 of the short peptides, PGN has been divided into two major types: L-lysine-type (Lys-type) and meso-diaminopimelic acid-type (Dap-type). The molecule recognizing and binding PGN is called PGN recognition protein (PGRP). Based on transcript length, PGRPs are classified into three categories: short PGRP (PGRP-S), long PGRP (PGRP-L) and intermediate PGRP (PGRP-I). All PGRPs (Mammalian PGPPs were named PGLYRPs) have at least one C-terminal PGRP domain (∼160 amino acids), sometimes two, that are structurally homologous to the bacteriophage T7 lysozyme, a Zn^{2+}-dependent amidase that hydrolyzes PGN (Liu et al., 2001), whereas their N-terminal regions are variable

Abbreviations: ChtBD, chitin binding; FITC, fluorescein isothiocyanate; GlcNAc, *N*-acetylglucosamine; LPS, lipopolysaccharide; LTA, lipoteichoic acid; MurNAc, *N*-acetylmuramic acid; PAMPs, pathogen-associated molecular patterns; PGN, peptidoglycan; PGRP, peptidoglycan recognition protein; PRR, pattern recognition receptor.

* Corresponding author. Address: Room 205, Ke Xue Guan, 5 Yushan Road, Ocean University of China, Qingdao 266003, China. Tel.: +86 532 82032787.
E-mail address: sczhang@ouc.edu.cn (S. Zhang).

0145-305X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.dci.2012.07.009

and specific for each type of PGRPs (see review by Ghosh et al., 2011), which has not been given sufficient attention.

PGRPs were first discovered in the silkworm *Bombyx mori* (Yoshida et al., 1996), and then identified in fruit fly, mollusks, echinoderms, and vertebrates including mice and humans (see review by Guan and Mariuzza, 2007; Dziarski and Gupta, 2010). Among them, the function and structure of insect and mammalian PGRPs are best characterized. Although crystal structures of insect and mammalian PGRPs are largely similar (Cho et al., 2007; Guan et al., 2004; Lim et al., 2006), they have apparently developed different functions. For example, insect PGRPs are involved in the Toll and Imd signal transduction pathways that lead to generation of antimicrobial peptides (Michel et al., 2001; Bischoff et al., 2004; Choe et al., 2002; Gottar et al., 2002; Ramet et al., 2002; Garver et al., 2006; Takehana et al., 2004), while mammalian PGRPs do not act through host signaling pathways but are directly microbicidal (Dziarski et al., 2003; Guan and Mariuzza, 2007; Lu et al., 2006; Royet and Dziarski, 2007; Tydell et al., 2006).

Despite the enormous progress in insect and mammalian PGRPs, they are just beginning to be characterized in other species, and little is known about their functions, although most of them are supposed to have amidase activity (see review by Dziarski and Gupta, 2006). Additionally, except for insect and mammalian PGRPs, structural and functional insights into PGRPs in other animals are rather limited.

Amphioxus or lancelet, a cephalochordate, occupies a nodal position bridging from invertebrates to vertebrates (see review by Stach, 2008). It lacks vertebrate-like adaptive immunity, but has developed an extraordinary germ-line-encoded diversity of innate immunity (Huang et al., 2008). Hence amphioxus is an important reference to the evolution of vertebrate immunity (see review by Zhang et al., 2009). A search of the recently completed draft assembly and automated annotation of the Florida amphioxus *Branchiostoma floridae* genome revealed the presence of total 16 PGRP genes (http://www.genome.jgi-psf.org//Brafl1/Brafl1.home.html), but little is known about their role and structure–activity relation. This study was therefore undertaken to examine the expression and bioactivity of a novel member of short PGRPs isolated from the amphioxus *Branchiostoma japonicum*, with a special emphasis on its N-terminal function.

2. Materials and methods

2.1. Cloning and sequencing of amphioxus PGRP-S cDNA

Total RNA was extracted with RNAiso Plus (TaKaRa, Dalian, China) from adult *B. japonicum* collected during the breeding season (mid-June to mid-July) in the vicinity of Qingdao, China, and digested with RNase-free DNase (TaKaRa, Dalian, China) to eliminate the genomic contamination. The first-strand cDNA was synthesized with reverse transcription system (TaKaRa, Dalian, China) using oligo d(T) primer and used as template. cDNA fragments of amphioxus PGRP-S were amplified by polymerase chain reaction (PCR) with the primer pairs P1 and P2, and P3 and P4 (Table 1), that were designed on the basis of the PGRP gene sequences found in *B. floridae* genome database (http://www.genome.jgi-psf.org//Brafl1/Brafl1.home.html). The complete coding sequence was obtained by RACE with the BD SMART™ RACE cDNA amplification kit (Clontech, Beijing, China) according to the instructions. The gene-specific primer pairs P5 and P6, and P7 and P8 (Table 1) were used in RACE reactions for the cloning of 3′-end and 5′-end cDNAs, respectively. The clones obtained were sequenced and the overlapping regions were assembled. According to the cDNA sequence assembled, the full-length open reading frame (ORF) of amphioxus *pgrp* was obtained by PCR with primer pairs P9 and P10 and verified by sequencing.

2.2. Sequence analysis

The cDNA sequence obtained was analyzed for coding probability with the DNATools program. Sequence comparison against the GenBank protein database was performed using the BLAST network server at NCBI. The SMART program (http://www.smart.embl-heidelberg.de/) was used to predict the functional sites and domains in the deduced amino acid sequence, and SignalP 3.0 (http://www.cbs.dtu.dk/services/SignalP/) used to predict the signal peptide. Multiple protein sequences were aligned using the MegAlign program of the LASERGENE software suite (DNASTAR).

2.3. Genomic DNA isolation and PCR

Genomic DNA was extracted from *B. japonicum* using the TIANamp marine animals DNA kit (Tiangen, Beijing, China) and used as template for PCR. The primer pairs P9 and P12, and P11 and P10 (Table 1) were used to amplify the genomic DNA. The PCR products were verified by DNA sequence analysis. The overlapping sequences were assembled and used to delineate the genomic structure.

2.4. Quantitative real-time PCR (qRT-PCR)

Total RNAs were extracted with RNAiso Plus from the various tissues of *B. japonicum*, including the gill, notochord, muscle, hepatic caecum, hind-gut, testis, and ovaries. After digestion with RNase-free DNase to eliminate the genomic contamination, the RNAs were used to synthesize cDNAs with reverse transcription system using the random primer. The cDNAs synthesized were used as template for qRT-PCR performed on ABI 7500 real-time PCR system (Applied Biosystems, USA). The gene-specific primers P13 and P14 (Table 1) were used to amplify a product of 194 bp. The housekeeping gene β-actin was selected as a reference to standardize normalization.

2.5. Construction of expression vectors

The complete cDNA region encoding mature amphioxus PGRP-S with the signal peptide deleted was amplified by PCR with the upstream primer P15 (*Bam*H I site is underlined) and the downstream primer P17 (*Hind* III site is underlined) (Table 1). To determine N-terminal role, the N-terminal 86 residues (consisting of the ChtBD1 domain; see below) of amphioxus PGRP-S were deleted. The upstream and downstream primers used were P16 (*Bam*H I site is underlined) and P17 (*Hind* III site is underlined), respectively (Table 1). All PCR products were digested with *Bam*H I and *Hind* III, and subcloned into the plasmid expression vector pET28a (Novagen, Darmstadt, Germany) previously cut with the same restriction enzymes. The plasmids constructed were verified by sequencing, and designated pET28a/PGRP-S (full-length mature protein without the signal peptide), and pET28a/P86/250 (N-terminal 86 residues deleted, but PGRP domain retained), respectively.

2.6. Expression, purification and refolding of recombinant proteins

The cells of *Escherichia coli* BL21 were transformed with the plasmids pET28a/PGRP-S and pET28a/P86/250, respectively, and then cultured overnight in LB broth containing 50 μg/ml kanamycin. The cultures were diluted 1:100 with LB broth and subjected to further incubation until OD_{600} reached about 1.0. The expression of recombinant proteins was induced by adding isopropyl β-D-thiogalactoside (IPTG) to the cultures at a final concentration of 0.1 mM. The inclusion bodies were prepared by the method of Liu and Zhang (2009a), and analyzed on a 12% SDS-PAGE gel.

The inclusion bodies prepared from 600 ml cell culture were resuspended in 25 ml of buffer B consisting of 100 mM Tris, 500 mM NaCl, 8 M urea and 10 mM imidazole (pH8.0) and incubated at 4 °C

Download English Version:

https://daneshyari.com/en/article/2429498

Download Persian Version:

https://daneshyari.com/article/2429498

Daneshyari.com