Fish & Shellfish Immunology 56 (2016) 181–187

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

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

Short communication

Three novel C1q domain containing proteins from the disk abalone *Haliotis discus discus*: Genomic organization and analysis of the transcriptional changes in response to bacterial pathogens

S.D.N.K. Bathige [a], Navaneethaiyer Umasuthan [a], J.D.H.E. Jayasinghe [a], G.I. Godahewa [a], Hae-Chul Park [b, **], Jehee Lee [a, *]

[a] Department of Marine Life Sciences & Fish Vaccine Research Center, Jeju National University, Jeju Self-Governing Province, 63243, Republic of Korea
[b] Graduate School of Medicine, Korea University, Ansan, Gyeonggido, 15355, Republic of Korea

ARTICLE INFO

Article history:
Received 13 April 2016
Received in revised form
28 June 2016
Accepted 9 July 2016
Available online 12 July 2016

Keywords:
Haliotis discus discus
C1q domain containing (C1qDC) protein
Genomic structure
Immune response

ABSTRACT

The globular C1q (gC1q) domain containing proteins, commonly referred as C1q domain containing (C1qDC) proteins, are an essential family of proteins involved in various innate immune responses. In this study, three novel C1qDC proteins were identified from the disk abalone (*Haliotis discus discus*) transcriptome database and designated as AbC1qDC1, AbC1qDC2, and AbC1qDC3. The cDNA sequences of *AbC1qDC1*, *AbC1qDC2*, and *AbC1qDC3* consisted of 807, 1305, and 660 bp open reading frames (ORFs) encoding 269, 435, and 220 amino acids (aa), respectively. Putative signal peptides and the N-terminal gC1q domain were identified in all three AbC1qDC proteins. An additional predicted motif region, known as the coiled coil region (CCR), was identified next to the signal sequence of AbC1qDC2. The genomic organization of the *AbC1qDC*s was determined using a bacterial artificial chromosome (BAC) library. It was found that the CDS of *AbC1qDC1* was distributed among three exons, while the CDSs of *AbC1qDC2* and *AbC1qDC3* were distributed between two exons. Sequence analysis indicated that the AbC1qDC proteins shared <40% identity with other counterparts from different species. According to the neighbor-joining phylogenetic tree, the proteins were grouped within an invertebrate group with high evolutionary distances, which suggests that they are new members of the C1qDC family. Higher expression of *AbC1qDC1* and *AbC1qDC2* was detected in hepatopancreas, muscle, and mantle tissues compare to the other tissues analyzed, using reverse transcription, followed by quantitative real-time PCR (qPCR) using SYBR Green, whereas *AbC1qDC3* was predominantly expressed in gill tissues, followed by muscles and the hepatopancreas. The temporal expression of *AbC1qDC* transcripts in gills after bacterial (*Vibrio parahaemolyticus* and *Listeria monocytogenes*) and lipopolysaccharide stimulation indicated that *AbC1qDC*s can be strongly induced by both Gram-negative and Gram-positive bacterial species with different response profiles. The results of this study suggest that AbC1qDCs are involved in immune responses against invading bacterial pathogens.

1. Introduction

The C1q domain containing (C1qDC) proteins are members of a family of proteins that have a globular C1q (gC1q) domain of approximately 140 amino acid (aa) residues located at the carboxyl terminus [1]. The basic structure of the C1q domain is characterized by a compact, jelly-roll topology consisting of a 10-stranded β-sandwich made up of two 5-stranded, anti-parallel, β-sheets [2]. C1qDC proteins play essential roles in innate immunity, including activation of the complement system [3], mediation of cell migration [4], pathogen recognition, and phagocytosis of bacteria [5]. The extreme functional capacity of C1qDC proteins is due to its potent ability to recognize patterns of various ligands, including the following: lipopolysaccharides (LPS) and porins, from Gram-negative bacteria; envelope proteins of retroviruses; β-amyloid fibrils; phospholipids; apoptotic cells; and some acute phase

* Corresponding author. Marine Molecular Genetics Lab, Department of Marine Life Sciences, College of Ocean Science, Jeju National University, 66 Jejudaehakno, Ara-Dong, Jeju, 63243, Republic of Korea.
** Corresponding author.
E-mail addresses: hcpark67@korea.ac.kr (H.-C. Park), jehee@jejunu.ac.kr (J. Lee).

http://dx.doi.org/10.1016/j.fsi.2016.07.010
1050-4648/

reactants [6,7].

A wide range of C1qDC proteins have been identified and well characterized in vertebrates, including the products of 31 and 52 C1qDC genes from humans [8] and zebrafish [9], respectively. Recently, a number of invertebrate *C1qDC* genes were also identified and their roles in the immune response were found to include recognizing pathogens and pathogen-associated molecular patterns (PAMPs) [10–12], agglutinating microorganisms [13], and mediating cell migration [4]. Moreover, transcriptional modulation of *C1qDC* genes in response to microbial pathogenic infections was also investigated [14,15]. In addition to the host immune defensive roles, C1qDC proteins were found to play an essential role in cellular apoptosis [16,17] and germ cell development during embryogenesis [18]. Surprisingly, 337 and 168 *C1qDC* genes have been identified in *Crassostrea gigas* [19] and *Mytilus galloprovincialis* [15], respectively, although a few *C1qDC* genes have also been reported in other invertebrate models. However, the potential roles and details of mechanisms of invertebrate *C1qDC* genes are still not completely understood.

Disk abalone (*Haliotis discus discus*), an economically crucial gastropod species in the South Korean aquaculture industry, is widely distributed around the coastal area. The study of the structure and possible immune roles of *C1qDC* genes in disk abalone in response to invading microorganisms may provide better understanding of defense mechanisms and new insights into the primary complement cascade in mollusks. The main objectives of this study were as follows: (1) to characterize the full-length cDNA and genomic sequences of three putative orthologs of *C1qDC* genes; (2) to examine the expression pattern of each *C1qDC* gene in different tissues; and (3) to examine the temporal responses of *AbC1qDC*s after administration of LPS and live bacterial species including *Vibrio parahaemolyticus*, a causative agent of withering syndrome [20] and *Listeria monocytogenes*, a potential food born pathogen [21].

2. Materials and methods

2.1. Identification of abalone C1q domain containing gene sequences

Three sequences containing information for the C1q domain were identified from the cDNA sequence database constructed by applying the Roche 454 Genome Sequencer FLX System (GS-FLX™) as previously described [22]. The BLAST algorithm from NCBI (http://www.ncbi.nlm.nih.gov/BLAST) was utilized to confirm the genes, which were designated as *AbC1qDC1, AbC1qDC2*, and *AbC1qDC3*. The bacterial artificial chromosome (BAC) clones containing genomic sequence for each *C1qDC*s were isolated from a BAC library established in our laboratory [23], based on a PCR screening method using gene specific primers (Supplementary Table 1). The sequence of each BAC clone was determined using the Roche 454 Genome Sequencer FLX (GS-FLX™) system (Macrogen Inc. Korea).

2.2. Bioinformatics analysis

Nucleotide and putative amino acid sequences of AbC1qDCs were analyzed by means of DNAssist version 2.2 [24] and the BLAST tool [25]. The SignalP 4.1 server (http://www.cbs.dtu.dk/services/SignalP/) was used to determine the signal peptide sequence and the putative cleavage site of each AbC1qDC. *N*-glycosylation sites were predicted using the NetNGlyc 1.0 Server [26]. Characteristic domains or motifs were identified using the conserved domain database (CDD) in NCBI [27] and the Simple Modular Architecture Research Tool (SMART) program [28]. The percent identity and percent similarity between sequences were estimated using the Matrix Global Alignment Tool (MatGat) [29]. The ClustalW program version 2 [30] was used to analyze the multiple sequence alignment. A neighbor-joining (NJ) tree with thirty ortholog sequences was constructed using MEGA version 5.05 [31]. Bootstrap trials were replicated 5000 times to evaluate the topological stability of the NJ tree. The Spidey program (http://www.ncbi.nlm.nih.gov/IEB/Research/Ostell/Spidey/) was used to locate the correct position of the exons and introns in the genomic sequence of each *AbC1qDC*. Molecular 3D models of the C1q globular region of each AbC1qDCs were generated by submitting the deduced amino acid sequences to the I-TASSER online program [32]. Swiss-Pdb viewer version 4.0.1 [33] was used to visualize the 3D structures.

2.3. Experimental animals and immune challenges

Healthy disk abalones (average weight, 50 g) were obtained from the Youngsoo farm in Jeju and reared at the Marine and Environmental Research Institute of Jeju National University, Republic of Korea. Animals were maintained for adaptation in sand-filtered sea water (salinity of 34 ± 0.6‰ and temperature of 20 ± 1 °C) with continuous aeration for 1 week before the experiments. Six different types of tissues, including those from digestive tract, hemocytes, gills, mantle, muscle, and hepatopancreas, were dissected from four healthy disk abalones to examine tissue specific mRNA expression. Immune challenge experiments were conducted as previously described [34]. Briefly, 100 μL of *V. parahaemolyticus* (1×10^4 CFU/animal), and *L. monocytogenes* (1×10^4 CFU/animal) in saline (0.9% NaCl) were injected intramuscularly. As an immune stimulant, 100 μL of LPS (5 μg μL^{-1}, *Escherichia coli* 055:B5; Sigma-Aldrich, USA) dissolved in saline was administered. Disk abalones of the negative control group were injected with 100 μL of saline. The gills were dissected at different time points (0, 3, 6, 12, 24, 48, 72, and 120 h) from four abalones of each challenged groups and were then snap frozen and stored at −80 °C.

2.4. RNA isolation and quantification of AbC1qDCs' transcripts

Total RNA was extracted from tissues pooled from four disk abalones by using Tri Reagent™ (Sigma-Aldrich, USA). Purity of the RNA was assessed spectrophotometrically at 260 nm and 280 nm. Subsequently, the concentration of RNA was measured at 260 nm, and first-strand cDNA was synthesized using the PrimeScript™ first-strand cDNA synthesis kit (TaKaRa, Japan) as described in a previous report [22].

The quantification of *AbC1qDC* transcripts in disk abalone under normal physiological conditions and immune-challenged conditions were measured by quantitative, real time PCR (qPCR) using the Real Time System TP800 Thermal cycler Dice™ (TaKaRa, Japan) with the fluorescent active SYBR Green. Gene-specific primers (Supplementary Table 1), designed following MIQE guidelines [35], were used to amplify each gene. Disk abalone ribosomal protein L5 (GenBank accession: EF103443) was used as an internal calibrator and corresponding primers are listed in Supplementary Table 1. The qPCR mixture (15 μL) comprised 7.5 μL of 2 × SYBR® *Premix Ex Taq*™ (Takara, Japan), 0.6 μL of each gene-specific primer (10 pmol/μL), 4 μL of diluted cDNA, and 2.3 μL of PCR-grade water. The thermal cycling conditions were as follows: 95 °C for 10 s and 45 cycles each of 95 °C for 5 s, 58 °C for 20 s, and 72 °C for 20 s. To assess the specificity of target amplification, an additional cycle of 95 °C for 15 s, 60 °C for 30 s, and 95 °C for 15 s was used. The qPCR analysis was performed in triplicates and each Ct (cycle threshold) value of the *AbC1qDC*s was normalized to corresponding Ct values of ribosomal protein L5. The Livak method [36] was used to evaluate the relative mRNA expression of the *AbC1qDC*s and final data were