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Three complement component 1q genes from rock bream, *Oplegnathus fasciatus*: Genome characterization and potential role in immune response against bacterial and viral infections

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ABSTRACT

Complement component 1q (C1q) is a subcomponent of the C1 complex and the key protein that recognizes and binds to a broad range of immune and non-immune ligands to initiate the classical complement pathway. In the present study, we identified and characterized three novel C1q family members from rock bream, *Oplegnathus fasciatus*. The full-length cDNAs of C1q A-like (RbC1qAL), C1q B-like (RbC1qBL), and C1q C-like (RbC1qCL) consist of 780, 720 and 726 bp of nucleotide sequence encoding polypeptides of 260, 240 and 242 amino acids, respectively. All three RbC1qs possess a leading signal peptide and collagen-like region(s) (CLRs) in the N-terminus, and a C1q domain at the C-terminus. The C1q characteristic Gly-X-Y repeats are present in all three RbC1qs, while the CLR-associated sequence that enhances phagocytic activity is present in RbC1qAL (^{49}GEKGEP^{54}) and RbC1qCL (^{70}GEKGEP^{75}). Moreover, the coding region was distributed across six exons in RbCqAL and RbC1qCL, but only five exons in RbC1qBL. Phylogenetic analysis revealed that the three RbC1qs tightly cluster with the fish clade. All three RbC1qs are most highly expressed in the spleen and liver, as indicated by qPCR tissue profiling. In addition, all three are transcriptionally responsive to immune challenge, with liver expression being significantly up-regulated in the early phase of infection with intact, live bacteria (*Edwardsiella tarda* and *Streptococcus iniae*) and virus (rock bream iridovirus) and in the late phase of exposure to purified endotoxin (lipopolysaccharide). These data collectively suggest that the RbC1qs may play defense roles as an innate immune response to protect the rock bream from bacterial and viral infections.

1. Introduction

The complement system (CS) is composed of more than 35 proteins in plasma and on cell surfaces, and plays a vital role in clearing pathogenic microorganisms, as well as infected and otherwise damaged cells, from the host system [1,2]. The CS can be activated by three major signaling cascades, known as the classical, lectin and alternative pathways, which lead to various but interactive biological processes, such as inflammation, phagocytosis, lysis, and the adaptive immune response [3]. The classical pathway itself is activated upon recognition and binding of antibody–antigen complexes by the complement component 1 (C1) complex, which is composed of the C1q recognition molecule and four serine protease proenzymes, including two units of C1r and two units of C1s [4].

C1q has been characterized as the primary link between innate immunity driven by classical pathway and acquired immunity mediated by the immunoglobulin (Ig) components of antibody–antigen complexes, IgG and IgM [5]. Moreover, its activity has been demonstrated as crucial to a broad spectrum of immunological processes, including phagocytic removal of bacteria, retrovirus neutralization, apoptotic cell clearance, immune cell adhesion, and growth modulation of dendritic cells, B cells, and fibroblasts [6].

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C1q mediates these effects via strong, targeted binding to a diverse set of host- and pathogen-derived ligands, such as the lipopolysaccharide (LPS) and porin bacterial endotoxins, the envelop proteins of some retroviruses, various phospholipids, β-amyloid fibrils, apoptotic cells, and pentraxins [7]. The unique protein structure of C1q might likely mediate this diverse capacity to recognize and bind such a vast array of ligands.

The teleostean complement system has been partially characterized in common carp [8], and number of complement components have been identified including C1r/C1s, C3, C4, C5, Bf/C2, Factor I and MASP (mannose-binding lectin associated serine protease). Some studies have shown that the CS components and functions of mammals are similar to those in rainbow trout (a bony fish) [9] and the nurse shark (a cartilaginous fish) [10]. The C1q subcomponent, in particular, has been identified in nurse shark [11], channel catfish [12], zebrafish (C1qA, C1qB and C1qC) [13], and mandarin fish (C1qL1 and C1qL2) [14]. In addition, several studies of fish [14] and some other invertebrates [15] have demonstrated that C1q activity is important to immune defense mediated by the classical complement pathway against invading pathogens.

Rock bream (*Oplegnathus fasciatus*) is one of the most economically important fish species of Korean aquaculture. In recent years, significant production losses have been caused by outbreaks of pathogenic diseases and researchers have directed their efforts towards understanding the underlying pathogenic mechanisms and immune responses to create a healthier and sustainable industry environment. In this study, we attempted to characterize the full-length cDNA and genomic sequence of putative C1qs from rock bream. Our data on the temporal expression profile and transcriptional response in liver to infection by common pathogens, including *Edwardsiella tarda* and *Streptococcus iniae* bacteria and the rock bream iridovirus (RBIV), provide novel insights into the C1q-mediated defense mechanisms of rock bream.

2. Materials and methods

2.1. Rock bream cDNA library construction

The rock bream cDNA sequence database was previously established by our laboratory using the next-generation pyrosequencing-based Roche 454 Genome Sequencer FLX System (GS-FLX™) [16]. In brief, total RNA was extracted from healthy rock bream using the Tri Reagent™ (Sigma, USA) and processed with the FastTrack® 2.0 mRNA isolation kit (Invitrogen, USA). The Creator™ SMART™ cDNA library construction kit (Clontech, USA) and Trimmer cDNA normalization kit (Evrogen, Russia) were used to synthesize and normalize first-strand cDNA, respectively. Subsequently, sequencing was carried out on the GS-FLX™ Titanium instrument (DNA Link, Inc., USA).

2.2. BAC library and genomic sequence screening

The rock bream bacterial artificial chromosome (BAC) library previously generated by our laboratory consists of 92,160 clones arrayed on 240 384-well plates [17]. PCR-based screening with gene-specific primers for C1q identified a putative clone containing the gene of interest, and the BAC clone was sequenced by the Roche GS-FLX™ System.

2.3. Identification of C1q genes from the rock bream database

Three full-length C1q sequences were identified in the rock bream database by applying the Basic Local Alignment Tool (BLAST) in the National Center for Biotechnology Information (NCBI) web-based query system (http://www.ncbi.nlm.nih.gov/BLAST) with default algorithm parameters. Two of these sequences were identified from the cDNA library and the third one was obtained from the BAC library. Genomic sequences for all three C1q genes were obtained from the BAC library. The coding sequences were cloned with conventional molecular biology techniques and sequences were reconfirmed.

2.4. Sequence analysis of C1q genes and 3D modeling

The open reading frames (ORFs) and amino acid (aa) sequences of the putative C1q genes were analyzed using DNAssist software (version 2.2). The MatGat program was used to evaluate the percentage of identity and similarity of putative aa C1q sequences with known vertebrate sequences published in GenBank. Several programs were used to analyze the deduced aa sequences of the rock bream C1qs, including the expert protein analysis system (http://www.expasy.org), the motif scan Pfam hidden Markov models (Local models) (http://hits.isb-sib.ch/cgi-bin/PFSCAN) for conserved domains, the ClustalW2 (http://www.ebi.ac.uk/Tools/clustalw2/index.html) for pairwise and multiple alignments, the SignalP program (http://www.cbs.dtu.dk/services/SignalP/) for prediction of signal peptides, and the NetNGlyc 1.0 Server (http://www.cbs.dtu.dk/services/NetNGlyc/) for prediction of potential N-glycosylation sites. The Spidey program (http://www.ncbi.nlm.nih.gov/IEB/Research/Ostell/Spidey/) was used to identify the exon-intron regions in the genomic sequence. Homologous genomic structures were obtained from the Ensembl genome browser (http://www.ensembl.org/index.html). Analysis of the genetic distances between C1q orthologes was carried out by constructing a phylogenetic tree using the neighbor-joining (NJ) method in MEGA software, version 5.05 (http://www.megasoftware.net). The 3D models of the rock bream C1q globular domains were constructed by the Swiss-Model protein modeling server [18] and manipulated with the Swiss-PdbViewer program, version 4.04; the model structures were based on the crystallographic structures of human C1q globular domains [19].

2.5. Animals, immune challenge experiments, and tissue collection

Healthy rock breams, averaging 50 g in body weight, were obtained from the Jeju Special Self-Governing Province Ocean and Fisheries Research Institute (Jeju, Republic of Korea) and acclimatized to laboratory conditions (400 L tanks with filtered sea water: salinity $34 \pm 0.6\%$, pH 7.6 ± 0.5, 23 ± 1 °C) for one week. For the live bacterial challenge experiments, each rock bream was intraperitoneally (i.p.) injected with 100 μL of Gram-negative *E. tarda* (5×10^3 CFU/μL) or Gram-positive *S. iniae* (1×10^5 CFU/μL), suspended in phosphate-buffered saline (PBS). These two bacterial strains were obtained from the Department of Aqualife Medicine at Chonnam National University (Republic of Korea) and grown in brain heart infusion broth (BHI). For the live virus challenge experiment, 100 μL of RBIV (10^3 $TCID_{50}$ of RBIV per fish) in PBS was injected [20]. For the mitogen stimulation experiment, 100 μL of purified LPS (1.25 μg/μL of *Escherichia coli* 055:B5; Sigma) in PBS was i.p. injected into each fish. A negative injection control group was established by i.p. injection of 100 μL PBS alone. Non-injected fish served as complete negative controls. Three random fish were sacrificed from all challenge and control groups for collection of liver samples at post-injection (p.i.) hours 3, 6, 12, 24 and 48 h ($n = 3$). Harvested tissues were snap-frozen in liquid nitrogen and stored at −70 °C.

2.6. Extraction of total RNA and synthesis of first-strand cDNA

Liver, gill, spleen, head kidney, kidney, blood, skin, muscle, heart, brain, and intestine were harvested from healthy fish to evaluate the normal tissue distribution pattern of the rock bream