Fish and Shellfish Immunology 76 (2018) 68–77

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

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

Full length article

A conserved interferon regulation factor 1 (IRF-1) from Pacific oyster *Crassostrea gigas* functioned as an activator of IFN pathway

Mengmeng Lu[a,c], Chuanyan Yang[a,c], Meijia Li[a,c], Qilin Yi[a,c], Guangxia Lu[a,c], Yichen Wu[a,c], Chen Qu[a,c], Lingling Wang[a,b,c,*], Linsheng Song[a,b,c]

[a] Liaoning Key Laboratory of Marine Animal Immunology, Dalian Ocean University, Dalian 116023, China
[b] Laboratory of Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266235, China
[c] Liaoning Key Laboratory of Marine Animal Immunology & Disease Control, Dalian Ocean University, Dalian 116023, China

ARTICLE INFO

Keywords:
Crassostrea gigas
Interferon
Interferon regulation factor
Transcriptional activation
Antiviral immunity

ABSTRACT

Interferon regulatory factors (IRFs), a family of transcription factors with a novel helix-turn-helix DNA-binding motif, play important roles in regulating the expression of interferons (IFNs) and IFN-stimulated genes. In the present study, an interferon regulation factor 1 was identified from oyster *Crassostrea gigas* (designated *Cg*IRF-1), and its immune function was characterized to understand the regulatory mechanism of interferon system against viral infection in invertebrates. The open reading frame (ORF) of *Cg*IRF-1 was 990 bp, encoding a polypeptide of 329 amino acids with a typical IRF domain (also known as DNA-binding domain). The mRNA transcripts of *Cg*IRF-1 were detected in all the tested tissues with the highest expression level in hemocyte. *Cg*IRF-1 protein was distributed in both nucleus and cytoplasm of the oyster hemocyte. The mRNA expression of *Cg*IRF-1 in hemocytes was significantly up-regulated at 48 h after poly (I:C) stimulation ($p < 0.05$). The recombinant *Cg*IRF-1 (r*Cg*IRF-1) could interact with classically IFN-stimulated response elements (ISRE) *in vitro*. The relative luciferase activity of interferon-like protein promotor reporter gene (pGL-*Cg*IFNLP promotor) was significantly ($p < 0.05$) enhanced in HEK293T cell after transfection of *Cg*IRF-1. These results indicated that *Cg*IRF-1 could bind ISRE and regulate the expression of *Cg*IFNLP as a transcriptional regulatory factor, and participated in the antiviral immune response of oysters.

1. Introduction

Interferon (IFN) system plays a key role in the non-specific immune response, especially against virus infection in vertebrates [1]. IFNs belong to class II helical cytokines family with pleiotropic biological activities. They have been demonstrated to play crucial roles in host innate and adaptive immunity in vertebrates [2]. Several viruses or microbial components (genomic DNA, single-stranded RNA (ssRNA), double-stranded RNA (dsRNA), RNA with 5′-triphosphate ends and viral proteins) could induce the IFN expression [3,4]. Subsequently, IFNs activate the Janus kinase signal transducer and activator of transcription (JAK-STAT) pathway by binding IFN receptor on the surface of immune cell to induce the expression of interferon stimulated genes (ISGs), and ultimately provoke the immune cells converting to be of antiviral potential [5,6]. In vertebrates, IFNs are divided into three types according to their structural features and functional differentiation [7]. The transcriptional activation of IFNs is regulated by interferon regulatory factors (IRFs) [8].

IRFs constitute a family of transcription factors which play important roles in regulating the expression of IFNs and IFN-stimulated genes [9]. To date, there are totally eleven members (from IRF-1 to IRF-11) identified from vertebrates [10]. All the IRF members share a well-conserved N-terminal helix-turn-helix IRF superfamily domain (also called DNA-binding domain, DBD) with five conserved tryptophan (Trp) residues, which could recognize DNA sequences containing 5′-GAAA-3′ tetranucleotide, such as the IFN-stimulated response element (ISREs, GAAANNGAAA) [11,12]. Moreover, it was reported that the IRS consensus (-78/-66, AANNGAAA), which existed in the promoter region of IFN-β, could be bound by the IRF family members [13]. As for the C-terminus, most of the IRFs share an IRF-3 superfamily domain, which was also named as IRF associated domain 1 (IAD1). IRF-1 and IRF-2 do not possess conserved IAD1, but they contain non-conserved activation domain (the last 100 amino acids of IRF-1 were rich in tyrosine) or repression domain (the final 25 amino acids of IRF-2 were rich in histidine, arginine and lysine) in their C-terminus, respectively. These two different domains are collectively termed IRF-associated domain 2

* Corresponding author. Liaoning Key Laboratory of Marine Animal Immunology, Dalian Ocean University, Dalian 116023, China.
E-mail address: wanglingling@dlou.edu.cn (L. Wang).

https://doi.org/10.1016/j.fsi.2018.02.024
Received 2 November 2017; Received in revised form 4 February 2018; Accepted 13 February 2018
Available online 17 February 2018
1050-4648/

(IAD2), also named as PEST (proline-, glutamic acid-, serine-, and threonine-rich) domain, which could mediate protein-protein interaction instead of IAD1 [14–16]. The structural features of DBD and IAD in IRFs determine their properties to perform different functions in IRF-dependent gene regulation.

The members of IRF family have been extensively investigated in vertebrates, and they exhibit diverse function in regulating antiviral response, immune cell growth, differentiation, and oncogenesis [17–20]. For instance, some IRFs (such as IRF-1, -3, -4, -5, -7, -8) are involved in antiviral immune response by regulating type I IFN and IFN-stimulated genes. IRF-1, -2, -4, and -8 are assigned to regulate the development or differentiation of immune cells (myeloid, DCs, NK, B, and T cells) in mammals [21]. IRF-1, -3, -5, and -8 possess tumor suppressor gene activities to regulate cell cycle or/and apoptosis [22]. Increasingly studies have proved versatile functions performed by this transcription factor family in vertebrates. For example, IRF-1 could interact with myeloid differentiation factor88 (MyD88) and significantly induce the expression of IFN gene involving in the IFN-mediated antiviral response, and it also exhibited roles in the development of NK and T cells, as well as the inhibition of cell growth [22–24].

Compared with the extensive knowledge of IRFs in mammals, research on IRFs in invertebrates and lower vertebrates are still in the infancy stage. A number of IRFs have been identified in lower vertebrates and invertebrates, including fish, arthropod and shellfish. A total of eleven IRFs have been found in fish, among which IRF-1 to IRF-9 are identified as conserved ones in comparison with those in mammals [25,26]. In amphioxus *Branchiostoma belcheri tsingtauense*, nine IRFs (named *bbt*IRF-1 to *bbt*IRF-9) have been identified, and *bbt*IRF-1 and *bbt*IRF-8 are found to be the well conserved orthologs of vertebrate IRF1 subgroup (IRF1SG) and IRF4SG [27], respectively. So far, four IRFs have been identified from pearl oyster *Pinctada fucata* (*Pf*IRF-2), pacific oyster *Crassostrea gigas* (*Cg*IRF-2 and *Cg*IRF-8) and pacific white shrimp *Litopenaeus vannamei* (*Lv*IRF) [28–30]. The IRFs in fish or invertebrates display abundant diversity, and some novel IRFs are found to be of species specific. For instance, IRF-11, firstly identified in zebrafish, is specific to teleost fish [31]. *Bbt*IRF-3, -5, -6, and -7 were unique in amphioxus [27]. The invertebrate IRFs share conserved N-terminal DBD, but their C-terminal IAD1 or IAD2 are significantly different [32]. The nine *bbt*IRFs in amphioxus are conserved in intron/exon organization compared with that of vertebrates, but different in the patterns of their C-terminal IAD1 splice junction [27]. *Bbt*IRF-1 was found to bind ISRE and recognize the promoter of human IFN-α1, IFN-α2, IFN-α6, and IFN-β [27]. However, there are few studies on the function of invertebrate IRFs, and their origin and evolution require further investigation.

Pacific oyster *Crassostrea gigas* is one of the important aquaculture bivalves worldwide. The cultured oysters can be infected by Ostreid herpes virus 1 in the marine environments, and the frequently outbreak of disease has threatened the sustainable development of oyster aquaculture [33–36]. As one of the most important antiviral lines in vertebrates, the existence of IFN system-mediated non-specific antiviral responses in invertebrates has been gradually confirmed [30,37–39]. With the release of oyster genome sequence of *C. gigas*, several key elements of the IFN pathway have been found [40], including an IFN-like protein (*Cg*IFNLP), an IFN receptor (*Cg*IFNR-3), and two IRFs [30,40–42]. It was found that the expression of two oyster IRFs could be induced by poly (I:C) stimulation. Moreover, *Cg*IRF-2 and *Cg*IRF-8 could activate the transcription of human IFNβ and ISRE promoters in mammalian cells [30]. In the present study, another novel IRF-1 homology (designed *Cg*IRF-1) was identified from *C. gigas* with the objectives (1) to investigate its mRNA distribution in tissues and the alternation of mRNA expression in the hemocytes after poly (I:C) stimulation, (2) to determine its subcellular localization in oyster hemocytes, (3) to validate the binding activity of recombinant *Cg*IRF-1 with classical ISRE sequence motif *in vitro*, and (4) to detect its transcriptional activity for downstream IFN-like protein (*Cg*IFNLP) in HEK293T cells, hopefully to provide information for the better understanding of IFN pathway and the antiviral mechanism of oyster.

2. Materials and methods

2.1. Oysters, immune stimulation and samples collection

Pacific oyster *C. gigas*, about two years old, was sampled from a commercial farm in Dalian, Liaoning province, China. They were acclimated in tanks with continuously aerated seawater at 15–20 °C for one week before experiment.

One hundred and eight oysters were randomly allocated to two groups (the treatment group and the control group) and kept in aerated tanks. Fifty four oysters in the treatment group received an injection of 100 μL poly (I:C) (1 mg mL^{-1} in seawater) (Sigma, USA, synthetic double stranded RNA), while the rest oysters in the control group received an injection of 100 μL seawater. After injection, the oysters were returned to aerated tanks and maintained under static conditions. Nine oysters were randomly sampled at 0, 6, 12, 24, 48 and 72 h post-injection from each group. The hemolymph from nine oysters was aseptically collected from the sinus of each oyster, and three oysters (three oysters in each parallel) were pooled together as one sample. The hemolymph was centrifuged at 800 *g*, 4 °C for 10 min to harvest the hemocytes. After addition of 500 μL Trizol reagent (Invitrogen), all these hemocyte samples were stored at -80 °C for subsequent RNA extraction.

Different tissues, including adductor muscle, gill, gonad, mantle, hepatopancreas, and hemocytes were obtained from nine oysters as parallel samples (three individuals in each parallel). The hemolymph samples were centrifuged at 800 *g*, 4 °C for 10 min to harvest the hemocytes.

2.2. RNA isolation and cDNA synthesis

Total RNA from different samples was extracted using Trizol (Invitrogen, USA) according to manufacturer's instruction. Briefly, about 50–100 mg samples were taken from different tissue of each oyster. After added 1 mL TRIzol™ Reagent, the samples were homogenized with lapping rod (hemocytes were broken by 1 mL syringe). Then 0.2 mL of chloroform was added into the tissue homogenate to separate total RNA. After precipitating the RNA using isopropanol, the total RNA was washed by 75% ethanol twice. Finally, the total RNA was resuspended by RNase-free water and prepared for cDNA synthesis.

The cDNA synthesis was performed using PrimeScript™ RT reagent Kit with gDNA Eraser (TaKaRa, Japan) according to the manufacturer's instruction. The first-strand cDNA synthesis was performed by primer oligo (dT) (P1 in Table 1), with the final concentration of 50 pmol. The total RNA (about 1 μg) was added into the 20 μL reaction system. Then, the mixture was incubated in 37 °C for 15 min, 85 °C for 15 s. Finally, the cDNA mix was diluted to 1:40 and stored at -80 °C for next processing.

2.3. Gene cloning and sequence analysis of CgIRF-1

Sequence information of *Cg*IRF-1 (XM_011451289/CGI_10021170) was searched from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) [40]. The open reading frame (ORF) sequence of *Cg*IRF-1 gene was amplified by a pair of gene specific primers (P2 and P3), and sequenced by primers P4 and P5 (Table 1). The resulting cDNA sequence and deduced amino acid sequence of *Cg*IRF-1 were analyzed by using BLAST algorithm (http://www.ncbi.nlm.gov/blast) and Expert Protein Analysis System (http://www.expasy.org/). The protein domain of *Cg*IRF-1 was predicted by using the simple modular architecture research tool (SMART) (http://smart.embl-heidelberg.de/). Multiple alignment analysis of *Cg*IRF-1 with other IRF superfamily members was performed with the ClustalW multiple alignment programs (http://www.ebi.ac.uk/clustalw/). A