Comparative Biochemistry and Physiology, Part B 223 (2018) 1–15

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part B

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

Molecular cloning and expression analysis of interleukin-1β and interleukin-1 receptor type I genes in yellow catfish (*Pelteobagrus fulvidraco*): Responses to challenge of *Edwardsiella ictaluri*

Chen-Lei Liao[a,b], Gui-Rong Zhang[a,b], Dong-Mei Zhu[a,b], Wei Ji[a,b], Ze-Chao Shi[b,c], Rui Jiang[a,b], Qi-Xue Fan[a,b], Kai-Jian Wei[a,b,*]

[a] *Key Laboratory of Freshwater Animal Breeding, Ministry of Agriculture, College of Fisheries, Huazhong Agricultural University, Wuhan 430070, PR China*
[b] *Freshwater Aquaculture Collaborative Innovation Centre of Hubei Province, Wuhan 430070, PR China*
[c] *Key Laboratory of Freshwater Biodiversity Conservation, Ministry of Agriculture, Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Wuhan 430223, PR China*

ARTICLE INFO

Keywords:
Bacterial challenge
cDNA cloning
Edwardsiella ictaluri
Gene expression
IL-1β
IL-1RI
Pelteobagrus fulvidraco

ABSTRACT

Interleukin-1β (IL-1β) is one of the pivotal early pro-inflammatory cytokines, which play important roles in regulating immune response and inducing a series of inflammatory reactions to infections. Interleukin-1 type I receptor (IL-1RI) is a receptor of the IL-1β that can mediate IL-1-dependent activation. In this study, partial cDNA sequences of the *Pf*_IL-1β and *Pf*_IL-1RI genes were cloned from yellow catfish (*Pelteobagrus fulvidraco*). The open reading frames (ORF) of *Pf*_IL-1β and *Pf*_IL-1RI genes encode putative peptides of 280 and 543 amino acids, respectively. The deduced amino acid sequences of these two genes shared highly conserved structures with those from other teleosts. Quantitative real-time PCR results showed that the *Pf*_IL-1β mRNA had relatively high expression levels in trunk kidney and blood, and the *Pf*_IL-1RI mRNA was highly expressed in blood and had relatively high expression level in liver. Ontogenetic expression analyses indicate that the *Pf*_IL-1β and *Pf*_IL-1RI genes may play important roles during the embryonic developmental stages. The mRNA expression levels of *Pf*_IL-1β and *Pf*_IL-1RI genes were up-regulated in the trunk kidney, head kidney, blood, spleen, heart and liver after *Edwardsiella ictaluri* challenge. Western blot analyses showed that *Pf*_IL-1β protein was highly expressed in the spleen and head kidney, but not in the fin of adult individuals. These results suggest that the *Pf*_IL-1β and *Pf*_IL-1RI genes may play significant roles in the immune regulation and defense against *E. ictaluri* in the yellow catfish.

1. Introduction

Interleukin 1 (IL-1) is a group of cytokines, which has a variety of biological effects on immunoregulatory and inflammatory activities (Maliszewski et al., 1988). Up to now, 11 members of the IL-1 family (IL-1α (IL-1F1), IL-1β (IL-1F2), IL-1 receptor antagonist (IL-1Ra/IL-1F3), IL-18 (IL-1F4), IL-1F5~10 and IL-33(IL-1F11)) have been identified in mammals (Garlanda et al., 2013). IL-1 family members share a broadly similar domain organization and receptor signaling pathways (Afonina et al., 2015).

In mammals, IL-1 is produced as a biologically inactive 31 kDa precursor, which is converted to the active 17 kDa mature form by the caspase-1 cleavage (Perregaux et al., 2002). IL-1β plays an important role in the host response to microbial invasion, tissue injury and immunological reactions including autoimmune diseases (Dinarello and Endres, 1989), and recombinant IL-1β is currently being studied or used as an adjuvant for vaccines in sheep (Lofthouse et al., 1995), pigs (Blecha, 1997) and cattle (Di et al., 2012).

IL-1β gene has been cloned from some species of teleosts, including salmonids (Ingerslev et al., 2006; Zou et al., 1999), cyprinids (Bo et al., 2015; Fujiki et al., 2000), gadoids (Corripio-Miyar et al., 2007; Seppola et al., 2008), Perciformes fish (Covello et al., 2009; Pelegrín et al., 2004) and Anguilliformes fish (Tsutsui et al., 2007), and has also been cloned from some cartilaginous fish (Bird et al., 2002; Secombes et al., 2011). In *Oncorhynchus mykiss*, the IL-1β was found to lack the classical IL-1β converting enzyme (ICE) cleavage site required for the maturation of mammalian IL-1β, and its gene expression could be induced by lipopolysaccharide (LPS) challenge in head kidney leukocytes and macrophages (Zou et al., 1999). This discovery provides evidence that fish IL-1βs possibly play important roles in the immune system similar to those in mammals.

* Corresponding author at: College of Fisheries, Huazhong Agricultural University, Wuhan 430070, PR China.
E-mail address: kjwei@mail.hzau.edu.cn (K.-J. Wei).

https://doi.org/10.1016/j.cbpb.2018.05.001
Received 6 February 2018; Received in revised form 5 May 2018; Accepted 10 May 2018

Interleukin-1 type I receptor (IL-1RI) is a kind of Toll-Interleukin family receptor. In mammals, the IL-1R family consists of 10 members that are receptors or co-receptors for the 11 ligands of the IL-1 family of cytokines (Boraschi and Tagliabue, 2013). The IL-1RI is the ligand-binding receptor of the receptor complexes for IL-1α and IL-1β thereby mediating IL-1-dependent activation (Sims and Dower, 1988). It has been reported that the IL-1RI may also bind IL-38 (IL-1F10). Ligand binding is brought about by the extracellular portion of the receptor molecule. Signaling depends on the cytoplasmic portion, about 200-amino acids-long, which includes a TIR domain (Narayanan and Park, 2015). In 1988, IL-1RI gene has been cloned from murine, then in the human in 1989. A number of incompletely spliced polyadenylated IL-1RI transcripts have been described in human monocytes stimulated with LPS, but its significance is unknown (Pentonrol et al., 1999; Spriggs et al., 1990).

In aquatic organisms, the IL-1RI gene has been cloned from some teleosts such as Atlantic salmon (*Salmo salar*), zebrafish (*Danio rerio*), and rainbow trout. Expression of Atlantic salmon IL-1RI gene was significantly increased in immune-related tissues after LPS stimulation, indicating that it is a cell specific regulation (Subramaniam et al., 2002).

Yellow catfish (*Pelteobagrus fulvidraco*) is an important commercial fish that is widely cultured in Asian countries, especially in China (Liu et al., 2010). With the rapid development of intensive farming in recent years, the yellow catfish culture industry has been severely affected by several kinds of bacterial diseases, including ascites disease, enteric septicemia and crack-head disease (Geng et al., 2010; Ye et al., 2009), and these diseases have led to heavy economic losses every year. In pond fish culture in China, yellow catfish was commonly infected with *Edwardsiella ictaluri*, which led to cutaneous hemorrhage, ulceration, enteritis and a soft, fluctuant red swelling on the dorsum of the head (Ye et al., 2009). Although outbreaks of diseases associated with bacteria have caused high mortality in farmed yellow catfish (Huang et al., 2012; Liu et al., 2016), information about its immune response is lacking.

In this study, we cloned the partial cDNA sequences of *Pf*_IL-1β and *Pf*_IL-1RI genes from the yellow catfish (*P. fulvidraco*) and detected the expression distribution of these two genes in various tissues from adults and the changes of mRNA expression of two genes during the embryonic and early larval stages. Subsequently we examined the changes of their expression in six immune-related tissues of juvenile yellow catfish after challenge with *E. ictaluri*. The expression analyses of the *Pf*_IL-1β and *Pf*_IL-1RI genes will help to better understand the potential immunomodulatory role of IL-1β and IL-1RI in yellow catfish against pathogenic microbes under physiological and pathological conditions.

2. Materials and methods

2.1. Fish collection, bacterial challenge and sampling

Eighty healthy adult individuals of yellow catfish (one year old) were obtained from the Jingzhou aquaculture base of Yangtze River Fisheries Research Institute, Hubei Province, China, and were transported to the fish breeding base of Huazhong Agricultural University (HZAU). Before artificial reproduction and gene cloning experiments, the fishes were acclimatized to laboratory conditions in two circulating water tanks by keeping the temperature at 26–28 °C and were fed a commercial diet (Hubei Haid Feeds Company, Wuhan, China) twice a day (08:00 and 17:00).

To clone the cDNA sequences of the *Pf*_IL-1β and *Pf*_IL-1RI genes and examine their expression distributions in various tissues, five adult individuals were sampled. The fish were anesthetized with tricaine methanesulfonate (MS-222, 300 mg/L) before dissection. Different tissues, including swim bladder, skin, midgut, liver, stomach, brain, gill, spleen, head kidney, muscle, heart, blood, fin and trunk kidney, were rapidly collected for RNA isolation. Nine tissues (liver, spleen, trunk kidney, head kidney, mucus, gill, midgut, skin and fin) were rapidly collected for protein extraction.

To detect the expression of the *Pf*_IL-1β and *Pf*_IL-1RI genes during the embryonic and early larval stages, three pairs of parent fish were selected for artificial propagation. Fertilized eggs from each pair were incubated separately in different indoor tanks with enough oxygen and a water temperature of 28–30 °C. After hatching, larvae were fed with yolk at three days post hatching (dph) and with fairy shrimp after 7 dph. Eight embryos and five larvae were randomly sampled from the same tank for RNA extraction at each of the following developmental stages: unfertilized egg, fertilized egg, multicellular stage, late blastula stage, late gastrula stage, neurula stage, somite appearance stage, muscular contraction stage, heart beating stage, prophase of hatching stage, newly hatched larvae, as well as larvae of 1/4, 1/2, 1, 3, 5, 7 and 10 dph.

To examine the immune responses of the *Pf*_IL-1β and *Pf*_IL-1RI genes after bacterial challenge, juvenile individuals of yellow catfish were collected at the fish breeding base of HZAU. The fish were reared in a circulating water tank for two weeks at 28–30 °C water temperature, with fish fed twice daily (08:00 and 17:00) with a commercial diet (Hubei Haid Feeds Company, Wuhan, China). The bacteria (*E. ictaluri*) for immune challenge experiments were obtained from the fish immunology and pathology laboratory of HZAU, and were cultured as described in the previous study (Wei et al., 2014). Fish were injected intraperitoneally with 200 μL of suspended *E. ictaluri* in phosphate buffered saline (PBS, pH 7.2) with a concentration of 1.5×10^7 CFU/mL as the experimental group or with the same volume of PBS as the control group. Five fish were randomly sampled from the experimental group at 6, 12, 24, 48, 72 and 120 hours post injection (hpi) and from the control group at each time point. The sampled fish were anesthetized with 300 mg/L MS-222, and then the trunk kidney, spleen, head kidney, heart, liver and blood tissues were collected for RNA extraction. The head kidney was collected for protein extraction.

All tissues, embryo and larvae samples were immediately frozen in liquid nitrogen and stored at −80 °C until RNA extraction or protein extraction. This study was approved according to the guidelines of the Institutional Animal Care and Use Committees (IACUC) of HZAU, Wuhan, P. R. China.

2.2. RNA extraction and cDNA synthesis

Total RNA was extracted from various tissues, embryos and larvae using TRIzol Reagent (Invitrogen, USA) according to the manufacturer's instruction. The quality of total RNA was checked by 1% agarose gel electrophoresis. The concentration of total RNA was determined using a Nanodrop ND-2000 spectrophotometer (Thermo Electrom Corporation, USA). The first strand cDNA was generated using the Revert Aid™ M-MLV Reverse Transcriptase Kit (Promega, USA) following the manufacturer's instructions. The cDNA products were stored at −80 °C.

2.3. Molecular cloning of the Pf_IL-1β and Pf_IL-1RI genes

To obtain the core sequences of the *Pf*_IL-1β and *Pf*_IL-1RI genes, two pairs of gene-specific primers (IL-1β-F1/IL-1β-R1 and IL-1RI-F1/IL-1RI-R1) were designed based on the previous transcriptome data of yellow catfish (Table 1). The PCR reactions were performed in a total volume of 20 μL, including 10 μL Premix *Taq* DNA polymerase (TaKaRa, Dalian, China), 2 μL (32 ng) cDNA, 0.5 μL (10 μM) of each primer and 7 μL ddH_2O. The PCR thermal cycling programs were set as follows: initial denaturation at 95 °C for 3 min; 35 cycles of 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 1–2.5 min; 72 °C for 10 min and 16 °C for 10 min.

To gain the 3′-untranslated region (UTR) sequence of the *Pf*_IL-1β gene, we designed two gene-specific primers according to the core cDNA sequence of the *Pf*_IL-1β gene, and performed the 3′ rapid amplification of cDNA ends (RACE) PCR with the link adapter (Table 1).