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Molecular cDNA cloning and analysis of the organization and expression of the IL-1β gene in the Nile tilapia, *Oreochromis niloticus*

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Abstract

The full-length cDNA sequence of interleukin-1β (IL-1β) from the Nile tilapia, *Oreochromis niloticus*, was determined by using PCR with primers designed from known fish IL-1β sequences followed by elongation of the 5′ and 3′ ends using Rapid Amplification of cDNA Ends (RACE). The cDNA contains a 92-bp 5′ untranslated region (UTR), a single open reading frame (ORF) of 732 bp that translates into a 243-amino acid molecule, a 341-bp 3′ UTR with four cytokine RNA instability motifs (ATTTA), and a polyadenylation signal (AATAAA) at 15 nucleotides upstream of the poly(A) tail. The organization of the genomic IL-1β based on the cDNA sequence appeared to be 4 introns and 5 exons. In comparison with known IL-1β amino acid sequences, including human, catshark, trout, turbot, carp, sea bream, sea bass and goldfish, the amino acid sequence deduced from the cDNA sequence of Nile tilapia showed different levels of identity ranging from 25.32% to 66.80% and homology ranging from 41.88% to 82.19%. Although the entire cDNA sequence of Nile tilapia IL-1β showed from 49.45% to 67.05% identity to those of other reported IL-1β cDNAs, each exon also showed different levels of identity to the corresponding exons of other reported IL-1β cDNAs. The highest nucleotide sequence identity for exon 1 and exons 2–5 of Nile tilapia IL-1β was found in the corresponding exons of sea bream and sea bass, respectively. After in vitro stimulation with lipopolysaccharide (LPS), we found an increased level of IL-1β expression in head kidney cells compared to that of unstimulated cells. However, this difference was no longer apparent after 4 h of stimulation, at which time the levels were similar in stimulated and unstimulated cells. Head kidney cells stimulated in vivo by an intraperitoneal injection of LPS showed a peak level of IL-1β expression after 1 day and a decreased level after 3 days. At 7 days after stimulation, we were hardly able to detect IL-1β expression.
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1. Introduction

Inflammation is one of the mechanisms of innate immunity and acts as a first line of defense against infection. Interleukin-1 (IL-1) is a major mediator of inflammation and stimulates and enhances the expression of several genes that are characteristically expressed during inflammation. IL-1, together with IL-1α, IL-1β, IL-1 receptor antagonist (IL-1ra) and IL-18 and a number of newly discovered genes (Smith et al., 2000), make up the IL-1 gene family in mammals (Dinarello, 1997).

In mammals, IL-1β is produced as an inactive precursor molecule that is processed by interleukin 1β-converting enzyme (ICE) (also called caspase-1) to give a biologically active peptide (Thornberry et al., 1992). There is considerable evidence that fish produce IL-1β during immune responses (Secombes et al., 1999). Over the last few years, bony fish and amphibian IL-1β have been extensively studied, and a number of regions that are conserved in vertebrates have been identified (Secombes et al., 1998; Zou et al., 2000). These discoveries allowed the cloning of the IL-1β genes from a number of teleost fishes.

To date the best-characterized IL-1β genes are those of the rainbow trout *(Oncorhynchus mykiss)* (Zou et al., 1999a) and carp (*Cyprinus carpio*) (Fujiki et al., 2000; Engelsma et al.,

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2001), although the IL-1β genes of the gilthead sea bream (*Sparus aurata*), sea bass (*Dicentrachus labrax*) and the small spotted catshark (*Scyliorhinus canicula*) have been sequenced (Pelegrin et al., 2001; Buonocore et al., 2003; Bird et al., 2002). Additionally, the in vitro biological activities of the IL-1 molecule suggested the possible use of IL-1β as an in vivo immuno-adjuvant in fish vaccination (Hong et al., 2001; Yin and Kwang, 2000).

The Nile tilapia, *Oreochromis niloticus*, is considered to be one of the most commercially important freshwater fish in Asian countries and therefore the study of a component of the innate immune system, IL-1β, of this fish will give a valuable insight into the control of diseases in aquatic industries. In the present study, we have determined the complete coding region and organization of the IL-1β gene in the Nile tilapia using PCR primers designed from the conserved regions of known fish IL-1β sequences. Studies of the modulation of IL-1β expression in response to in vivo and in vitro stimulation with bacterial LPS have also been made.

2. Materials and methods

2.1. Fish

Nile tilapia, *O. niloticus*, weighing 200–300 g were obtained from the aquatic farms of Pukyong National University and maintained at 22–25 °C in a 2-ton tank for at least 2 weeks prior to experimental use. The fish were fed twice daily on commercial pellets.

2.2. Cell preparation

For in vitro experiments, head kidney cells (1×10^8 cells/ml) were isolated from individual Nile tilapia by disrupting the tissue through a 100-μm nylon mesh. After washing in HBSS, the cells were suspended in L-15 medium (Gibco) and stimulated with 10 μg/ml (*Escherichia coli* O127:B8 lipopolysaccharide, Sigma-Aldrich, St. Louis, MO, USA) for 0, 0.5, 2.5, 4 h at 25 °C. Unstimulated cells were also cultured for several of the time periods shown above. To minimize the background levels of IL-1β expression induced by the isolation procedures, we did not use serum in the cell isolation medium, or a density gradient to enrich for particular leukocyte types.

For the in vivo experiments, 100 μg of LPS in 100 μl of phosphate-buffered saline (PBS) was injected intraperitoneally into each tilapia, and 1, 3, and 7 days later the head kidney cells were obtained as described above for experiments.

2.3. RNA isolation and IL-1β cloning

Head kidney cells (1×10^8 cells/ml) that had been stimulated with 10 μg LPS/ml for 4 h at 25 °C were centrifuged at 500×*g* and total RNA was extracted from the cell pellets with TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. cDNA was synthesized from 5 μg of total RNA by using Moloney murine leukemia virus reverse transcriptase at 42 °C for 45 min with an oligo-dT primer and was used as a template for PCR. The cDNA was used in the initial PCR with primers (IL1F-IL1R and IL1F-IL2R) derived from the reported rainbow trout and sea bream IL-1β sequences (IL1F and IL2R from the rainbow trout IL-1β sequence, GenBank accession number AJ223954, IL1R from the sea bream IL-1β sequence, GenBank accession number AJ277166). Amplification was performed in 50 μl reactions containing the following components: 1.25 μl of IL1F-IL1R and IL1F-IL2R primers (each 10 mM), 4 μl of dNTP mixtures (10 mM each), 11 μl of Taq polymerase buffer (10× containing 25 mM $MgCl_2$, Gencraft, Münster, Germany), 0.5 μl (2.5 units/μl) of Taq polymerase, 30.5 μl of sterile distilled H_2O and 1.5 μl of cDNA template. The cycling program was as follows: an activation step of 94 °C for 3 min, 30 cycles of 94 °C for 1 min, 55–60 °C for 1 min and 72 °C for 1.5 min, and an elongation step of 72 °C for 7 min. PCR products were visualized on a 1% agarose gel. After amplicons were purified (Gel Elution Kit, Bioneer, Daejeon, Korea), they were cloned into the TOPO-TA vector (Invitrogen) and transfected into competent *E. coli* DH-5α cells. Plasmid DNA was isolated and sequenced using the Big Dye Terminator Cycle DNA Sequencing Kit (ABI PRISM, PE Applied Biosystems, Foster City, CA, USA) and an automatic sequencer.

The full sequences of the 5′ and 3′ ends were obtained by rapid amplification of 5′ and 3′ cDNA ends PCR (RACE-PCR), using the specific primers (IL2F and IL1R) deduced from the partially determined nucleotide sequence of Nile tilapia IL-1β cDNA and the RACE kit primers following the manufacturer's instructions (Invitrogen) (Table 1). Nucleotide sequences and the deduced amino acid sequences were compared based upon a gene alignment using the BioEdit program (Version 7.0.6. Department of Microbiology, North Carolina State University, Raleigh, NC, USA). Based on the alignment, a phylogenetic tree was constructed using the Clustal W program (European Molecular Biology Laboratory, Heidelberg, Germany) and generated by the MEGA2 program (Version 2.1. Department of Biology, Arizona State University, Tempe, AZ, USA).

2.4. Genomic IL-1β cloning

Genomic DNA was isolated from fresh Nile tilapia kidney with a DNA extraction kit (Bioneer, Daejeon, Korea) following the manufacturer's instructions. Three separated PCRs were performed with different primer sets specific for IL-1β cDNA: T-ILF1/T-ILR1, T-ILF2/T-ILR2 and T-ILF3/T-ILR3 (Table 1). For each PCR, amplification was carried out in 50 μl reactions containing the following components: 1.25 μl forward and reverse primers (each 10 mM), 4 μl dNTP mixtures (10 mM each), 5 μl Taq buffer (10×, containing 25 mM $MgCl_2$, Gencraft), 0.5 μl (2.5 units/μl) Taq polymerase, 28 μl sterile distilled water and 10 μl (0.5 μg/μl) genomic DNA template. The cycling protocol was 1 activation step of 95 °C for 3 min, 30 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s followed by 1 elongation step of 72 °C for 7 min. PCR products were purified, cloned and then plasmid DNA was isolated and sequenced.

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