Molecular Characterization of Rockbream (*Oplegnathus fasciatus*)
Cytoskeletal β-actin Gene and Its 5'-Upstream Regulatory Region

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The cytoskeletal β-actin gene and its 5'-upstream region were isolated and characterized in the rockbream (*Oplegnathus fasciatus*). Complementary DNA of the rockbream β-actin represented a 1,125 bp of an open reading frame encoding 375 amino acids, and the rockbream β-actin cDNA and deduced amino acid sequences were highly homologous to those of other vertebrate orthologs. At the genomic level, the β-actin gene also exhibited an organization typical of vertebrate cytoskeletal actin genes (2,159 bp composed of five translated exons interrupted by four introns) with a conserved GT/AG exon-intron splicing rule. The putative non-translated exon predicted in the rockbream β-actin gene was much more homologous with those of teleostean β-actin genes than those of mammals. The 5'-upstream regulatory region isolated by genome walking displayed conserved and essential elements such as TATA, CArG and CAAT boxes in its proximal part, while several other immune- or stress-related motifs such as those for NF-kappa B, USF, HNF, AP-1 and C/EBP were in the distal part. Semi-quantitative RT-PCR assay results demonstrated that the rockbream β-actin transcripts were ubiquitously but differentially expressed across the tissues of juveniles.

Key words: Rockbream (*Oplegnathus fasciatus*), β-actin, Gene structure, mRNA expression, Regulatory region

Introduction

Cytoskeletal β-actin, a highly conserved protein found in a wide spectrum of animal cell types, plays crucial roles in maintaining cell shape and cellular mobility (Reece et al., 1992). Due to its constitutive and housekeeping expression, β-actin mRNA has been used commonly as an invariant standard for various gene expression assays (Pang and Ge, 2002; Andreassen et al., 2005; Cao et al., 2007). More importantly, the functional utility of the β-actin promoter as a strong regulator to drive heterologous expression of its downstream sequence has been reported in various fish species harboring the β-actin regulator-driven gene construct (Liu et al., 1990; Nam et al., 2001, Hwang et al., 2003; Brooks et al., 2007).

Genetic determinants of β-actins have been isolated and characterized from many teleost species. Previous studies have indicated that most fish β-actin genes share high sequence homology and had evolved from a common ancestor (Lee and Gye, 2001; Noh et al., 2003; Kim et al., 2008). However, barring a few studies, postmortem information on the isolation of fish β-actin genes has been limited mainly to the characterization of structural genes or coding regions. The regulatory regions of the fish β-actin genes, however, have been relatively less studied (see Kim et al., 2008). Structural and functional characterization of the 5'-flanking region in the β-actin gene is a prerequisite for employing the β-actin promoter as a regulatory element in germ-line transgenesis and DNA vaccination (Nam et al., 2008; Ruiz et al., 2008).

The objective of this study was to characterize the molecular structure of the cytoplasmic β-actin gene in the rockbream (*Oplegnathus fasciatus*), a highly valued marine food fish in Korea. We thus isolated and characterized rockbream β-actin cDNA and genomic DNA gene; assessed the structure and putative elements of the 5'-flanking regulatory region; and examined the tissue expression of β-actin transcripts.
in a semi-quantitative RT-PCR assay.

Materials and Methods

Isolation of rockbream β-actin cDNA

From our rockbream liver and kidney expressed sequence tag (EST) databases (unpublished data), clones exhibiting significant homology to previously known teleostean β-actin orthologs were surveyed. Based on a contig analysis with 20 selected clones (9 liver and 11 kidney clones) using Sequencher (Gene Codes, USA), the continuous version of full-length rockbream β-actin cDNA was isolated by RT-PCR. DNase-treated liver total RNA (2 μg) was reverse-transcribed using Omni-RTase (Qiagen, Germany) and oligo-d(T)20 primer according to the manufacturer’s recommendation. The RT-product (2 μl) was subjected to PCR amplification with primers (RB β-ACT 1F: 5’-TTCATTTGCCTGAAACCGGTTC-3’ and RB β-ACT 1R: 5’-CATGTCGGAACACATGTGCA-3’). Amplification of the expected 1,856-bp product was carried out using the Expand High Fidelity PCR System (Roche Applied Science, Germany) under the following thermal cycling conditions: 30 cycles at 94°C for 45 s, 58°C for 45 s and 72°C for 1 min with an initial denaturation step at 94°C for 3 min. The amplification product was cloned into pGEM T-easy vector (Promega, USA) and sequenced in both directions using the primer walking method.

Isolation of rockbream β-actin genomic gene

Based on the full-length cDNA sequence, the continuous fragment from ATG through the stop codon (TAA) was isolated by genomic PCR. Genomic DNA was purified from whole blood using the conventional SDS/proteinase K method and the integrity of the genomic DNA was confirmed by agarose gel electrophoresis. Next, 1 μg of the genomic DNA was subjected to PCR amplification using primers, RB gβ-ACT 1F (5’-TCAGCCATGGAAGATCGAAATCG-3’) and RB gβ-ACT 1R (5’-CATGTCGGAACACATGTGCA-3’). Amplification of the expected 1,856-bp product was carried out using the Expand High Fidelity PCR System (Roche Applied Science, Germany) under the following thermal cycling conditions: 30 cycles at 94°C for 45 s, 58°C for 45 s and 72°C for 1 min with an initial denaturation step at 94°C for 3 min. The amplification product was cloned into pGEM T-easy vector (Promega, USA) and sequenced in both directions using the primer walking method.

Isolation of the β-actin 5’-flanking upstream region

To amplify the proximal 5’-flanking region of the rockbream β-actin gene, a forward primer, RB β-ACT 5FW (5’-TCAGCCATGGAAGATCGAAATCG-3’) and reverse primer, RB gβ-ACT 2R (5’-ACCTCATTGGACTGCATAGC-3’), was designed to be complementary to the first translated exon of the rockbream β-actin gene. PCR amplification (30 cycles at 94°C for 1 min, 58°C for 1 min and 72°C for 2 min with an initial denaturation step at 94°C for 4 min) was carried out using the Expand High Fidelity PCR system. PCR products were cloned into pGEM T-easy vector, and insert sequences were obtained as described above. Based on the sequence information, the distal region of the β-actin upstream sequence was obtained by the genome walking method. Genome walking was performed with the GenomeWalker Universal Kit (Clontech Laboratories, USA) according to the manufacturer’s instructions. Briefly, 2.5 μg of genomic DNA was digested by EcoRV, DraI, PvuII or Stul restriction endonuclease. After the adaptor ligation step as guided by the instruction manual, two rounds of successive PCR amplifications were carried out using one each of the two primer pairs AP1 (forward primer provided in the kit) and RB β-actin GW01 (5’-GTGACGGCAGCCTAGATGGAAGATCGAAATCG-3’) and AP2/ RB β-actin GW02 (5’-GTGGCCACTGTTTTTATACGGCGCTCATTAGGACTGCATAGC-3’). Amplified products were TA cloned and sequenced as above. Based on the contig assembly, a continuous version of the 5’-flanking region was isolated again from the genomic DNA and its sequence was confirmed.

Sequence analysis

The amino acid sequence was deduced using the open reading frame (ORF) finder by NCBI (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). Multiple sequence alignments of the nucleotide and amino acid sequences were performed using ClustalW (http://align.genome.jp). Transcription factor binding motifs in the 5’-flanking upstream region of rockbream β-actin were predicted using TESS (http://www.cbil.upenn.edu/cgi-bin/tess/tess?RQ=WELCOME) and the TFSEARCH (http://www.cbr.jp/research/db/TFSEARCH.html). The rockbream β-actin cDNA and genomic sequences are available from GenBank under accession numbers FJ975145 and FJ975146, respectively. To perform the molecular phylogenetic analysis of the putative non-translated exon (first exon) from the teleostean β-actin genes, publically available β-actin sequences were either downloaded from GenBank or deciphered from the Ensembl genome database (http://www.ensembl.org/index.html). Putative nontranslated exon I sequences were aligned using ClustalW with the default setting for gap penalties in BioEdit 7.0.5 (Hall, 1999). The