Rapid communication: Nucleotide sequence of red seabream, *Pagrus major*, β-actin cDNA

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Name of the Sequence. Red seabream β-actin cDNA.

Genus and Species. *Pagrus major*.

Origin of Clone. Total RNA was isolated from ovary of red seabream. Poly(A)+ RNA was purified using an Oligotex-dT30 (Super) mRNA purification kit (Takara, Japan). Double-stranded complementary DNA synthesis was carried out using a TimeSaver cDNA synthesis kit (Pharmacia Biotech, Uppsala, Sweden). The cDNA library was constructed with a Lambda gt10/EcoRI/CIAP-treated vector (Stratagene, La Jolla, CA). To target β-actin cDNA from red seabream, primers for PCR were synthesized referring to DNA nucleotide sequences reported for medaka β-actin (Takagiri et al., 1997). The PCR primers used to screen the red seabream ovary cDNA library were ACTACCTCATGAAGATCCTG for the forward primer and TTGCTGATCCAATCTGCTG for the reverse primer. Reverse transcriptase-PCR amplified a 517-bp band from red seabream cDNA synthesized from the ovary RNA. The PCR product as a probe was labeled with [α-32P]dCTP (Oligo-labelling kit, Pharmacia Biotech) to screen the cDNA. The positive clones containing the insert were digested with Sau3AI, SacI, and ScaI and then subcloned into pBlue Script SK+. Both strands were sequenced with an ALFexpress DNA Sequencer System (Pharmacia Biotech).

Comparison with Related Sequences. The deduced amino acid sequence of red seabream β-actin showed 96.3% identity to both human (Ponte et al., 1984) and medaka (Takagiri et al., 1997) and 95.7% identity to gilthead seabream β-actin (Santos et al., 1997).

Sequence Data. The red seabream β-actin cDNA clone contained 1,521 nucleotides (nt), including the open reading frame that encoded 375 amino acids. The stop codon was followed by a 3′ untranslated region of 342 nt, including the polyadenylation signal, AATAAA. DDBJ/EMBL/GenBank Accession Number. AB036756.

Comments. From the total of 375 amino acids, the human β-actin amino acid sequences differed by 7 amino acids and 14 amino acids from gilthead seabream and red seabream β-actin amino acid sequences, respectively (Figure 1). The amino acid sequences of red seabream β-actin differed from those of β-actin of gilthead seabream, which belongs to the same family as red seabream, by 16 amino acids out of 375. Comparison of the deduced amino acid sequence of the red seabream β-actin gene was found to be highly conserved in relation to other known actins. However, the red seabream β-actin isoform has a higher homology to human β-actin than β-actin of gilthead seabream, which belongs to the same family as red seabream. Therefore, the possibility that red seabream β-actin and gilthead seabream β-actin have different isoforms must be considered.

Literature Cited


Key Words: Actin, Complementary DNA, DNA Cloning, Red Seabream

Figure 1. The amino acid sequences were extracted from the DDBJ/EMBL/GenBank sequence databases. Comparison of the amino acid sequences encoded by the red seabream β-actin (RsACT) (this paper, AB036756) with gilthead seabream (GsACT) (X89920), medaka (MeACT) (D89627), and human β-actin (HuACT) (84144061). The amino acid sequences were optimally aligned so as to match identical residues, which are indicated by asterisks.