Molecular cloning and characterization of ornithine decarboxylase gene from flounder (*Paralichthys olivaceus*)

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Abstract

Ornithine decarboxylase (ODC) is the key enzyme in the synthetic pathway of polyamines. This enzyme is a homodimeric and a pyridoxal 5-phosphate (PLP) dependent enzyme. We have isolated, a cDNA clone encoding ODC from brain cDNA library constructed from flounder (*Paralichthys olivaceus*). The ODC cDNA contained a complete ORF consisting of 460 amino acids and one stop codon with comparison to nucleotide sequences of the flounder, zebrafish and rat ODC genes, the ODC genes were highly conserved. The transcription of ODC was analyzed with reverse transcription-polymerase chain reaction (RT-PCR) species in brain, kidney, liver, and embryo.

Key words: Ornithine decarboxylase (ODC), Pyridoxal 5-phosphate (PLP), flounder

Introduction

Intracellular polyamine concentrations are highly regulated by the enzyme ornithine decarboxylase (ODC), which catalyzes the conversion of ornithine to putrescine. The eukaryotic ODCs are pyridoxal-5-phosphate (PLP) dependent enzymes.[3,4] The ODC genes have been cloned from various species. In the present study, we initially focus on the isolation and characterization of cDNAs. Herein, we provide the molecular characteristics and the pattern of tissue expression of the flounder ODC cDNA.

Materials and Methods

Screening of ODC cDNA and DNA sequencing

Total RNA was isolated with a TRIzol reagent (Invitrogen). The construction of the brain cDNA library was performed using a ZAP-cDNA® Synthesis Kit (Stratagene). DNA

sequencing was performed and determined with ABI 377 Genetic Analyzer according to the manufacturers instructions (Applied Biosystems).

Comparative sequence analysis of flounder ODC

To define the molecular evolution of ODC, several vertebrates ODC sequences were imported from the SwissPort data bank/GenBank and all the sequence data were analyzed using the Internet-based programs such as BLASTN and BLSTX program in the GeneBank database. A phylogenetic dendrogram presented by means of the Treeview program.

Reverse transcription-polymerase chain reaction (RT-PCR)

In order to perform RT-PCR, total RNA was isolated from the tissues of flounder in brain, kidney, muscle, liver, and embryo.

Results and discussion

In the present study, the cDNA encoding flounder ODC was cloned and its nucleotide sequence was determined. The flounder ODC has a 2939 bp fragment carrying the entire ORF (1380 nucleotides), 5'-noncoding region (312 nucleotides), and 3'-noncoding region (1247 nucleotides). The 3'-UTR has contained polyadenylation signals (attaaa). The flounder ODC have a high similarity in amino acid residues with other species, greater than 60% sequence identity. By this analysis flounder ODC shows 80.3% and 70.6% sequence identity with zebrafish and human ODC, respectively. The conservation of the ODC amino acid sequence among different species is high and the identity. The highest level of similarity concerns residues responsible for active site formation and stabilization of the ODC dimmer, which are almost identical among species. Structural elements involved in regulation of ODC translation and degradation of the enzyme (antizyme binding element of the ODC protein and its C-terminal end) are diverse. The flounder ODC has a putative N-linked glycosylation site (N-X-S/T) (residues 29-31; 71-73). Flounder ODC has a distinguished domain in the c-terminus interestingly when it aligned with ODCs from other species. In other to characterize the tissue expressions of flounder ODC, the reverse transcription-polymerase chain reaction (RT-PCR) was performed and the results showed that the expression of ODC gene was detected in the tissues of embryo, brain, young brain, kidney, and liver.

Summary

The flounder ODC has a 2939 bp fragment carrying the entire ORF (1380 nucleotides) and shares a high similarity in amino acid residues with other species. The patterns of tissue expressions of flounder ODC, RT-PCR and its expression was detected in brain, kidney, and liver. Herein, we provided the molecular characteristics and tissue expressions of the flounder ODC cDNA from adult flounder.

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