Isolation and Characterization of the Ornithine Decarboxylase gene from Flounder (*Paralichthys olivaceus*)

넙치 Ornithine decarboxylase 유전자의 분리 및 특성 연구

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A Dissertation

by

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ABSTRACT

Ornithine decarboxylase (ODC) is a pyridoxal 5'- phosphate (PLP)-dependent homodimeric enzyme and catalyzed the rate-limiting step in the polyamine biosynthetic pathway. Polyamines are essential for cell growth and function. The cDNA clone corresponding to ODC from the brain of adult flounder (*Paralichthys olivaceus*) is identified. The nucleotide and deduced amino acid sequences of the flounder ODC cDNA revealed an open reading frame of 1380 bp that corresponded to a protein of 460 amino acids residues, with a calculated molecular mass of 50

kDa. The flounder ODC shows 80.3% sequence identity to zebrafish and 70.8% to rat at the amino acid level, respectively. Structure and nucleotide sequence of ODC genes were compared and ODC gene is highly conserved among the species. The presence of ODC mRNA species in brain, kidney, liver, and embryo was confirmed using the reverse transcription- polymerase chain reaction (RT-PCR). A recombinant protein of flounder ODC containing a short histidine tag at the carboxyl-terminus was overexpressed in *Escherichia coli* BL21 (DE3) codon plus using an inducible T7 expression system, and the expressed protein was purified by Ni-NTA affinity chromatography.

Key words ; Ornithine decarboxylase (ODC), Pyridoxal 5'-phosphate (PLP), flounder (*Paralichthys olivaceus*)

INTRODUCTION

The polyamines such as putrescine, spermidine, and spermine are small polycations. The polyamines are essential for cell growth, and function such as embryonic development, stabilization of chromatin, translation, transcription, DNA replication and the protection of cells from DNA damage (Pendeville *et al*, 2001). The pathway of polyamine synthesis is shown in Figure 1. However, the excessive levels of polyamines may have toxic effects. Intracellular polyamine concentrations are highly regulated by the enzyme ornithine decarboxylase (ODC). ODC catalyzes the conversion reaction of ornithine to putrescines. This step is the first and the rate limiting step in polyamine biosynthesis (Cohen, 1998; Heby *et al*, 1990; Thomas and Thomas, 1984). The eukaryotic ODCs are a pyridoxal 5'-phosphate (PLP)-dependent homodimeric enzyme (Osterman *et al*., 1995b; Kern *et al*., 1999). The active sites are formed at the interface between the N-terminal domain of one monomer, which provides residues involved in PLP-interactions, and the C-terminal domain of the other subunit (monomer) providing residues previously identified as interacting with substrate (Almrud *et al*, 2000).

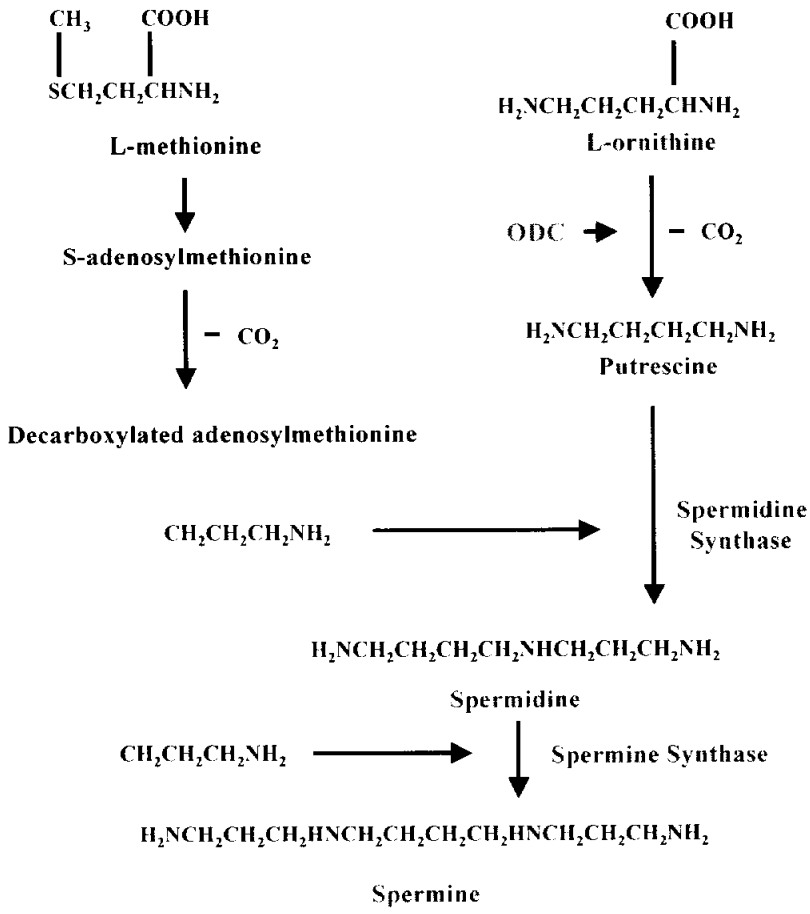

Figure 1. The polyamine biosynthetic pathway

Putrescine is formed by the decarboxylation of ornithine by ornithine decarboxylase. Spermidine is formed by the action of spermidine synthase that links putrescine to an aminopropyl group derived from decarboxylated S-adenosylmethionine, a reaction product of S-adenosylmethionine decarboxylase. Spermine is synthesized from spermidine by a similar process by spermine synthase (Thomas *et al*, 2001).

ODC is one of the most highly regulated enzymes (Holm *et al* 1998; Heby and Persson, 1990) and has a short half-life. Therefore ODC is a good target for studies on the selective proteolysis mechanism. The ODC activity is tightly regulated at the transcriptional and translational levels, as well as at the level of mRNA and protein stability (Murakami *et al.*, 1985; Katz and Kahana, 1987; Persson, 1988; Grens and Scheffler, 1990; Chen and Chen, 1992). The enzyme may be down-regulated by polyamines (Pegg, 1986), which exert most effective repression through antizyme (Coffino, 2001). Antizyme is an ODC inhibitory protein that is induced by polyamine-dependent frame-shift mechanism (Matsufuji *et al*, 1995). It inhibits ODC and triggers its degradation by the 26S proteasome (Coffino, 2001; Hayashi and Murakami, 1995; Hayashi *et al*, 1996), possibly after a conformational change in the ODC monomer (Coffino, 1998; Almrud *et al*, 2000; Murakami *et al*, 2000). The degradation of ODC occurs without ubiquitination in an ATP-dependent manner.

In normal cell, ODC expression is tightly regulated, whereas in tumor cell, ODC expression is abnormally regulated. Therefore, ODC is one of important genes during the early stages of tumor progression. ODC cDNAs have been cloned from *Homo sapiens* (human, Moshier *et al.*, 1990), *Bos taurus* (cow, Yao *et al.*, 1995), *Mus musculus* (house mouse, Kahana and Nathans, 1985), *Rattus norvegicus* (Norway rat, van Kranen *et al*, 1987),

Cricetulus griseus (Chinese hamster, Grens *et al.*, 1989)), *Mus pahari* (shrew mouse, Johannes and Berger, 1992), *Gallus gallus* (chicken, Johannes and Bulfield, 1992), *Trypanosoma brucei* (Phillips *et al.*, 1987), *Drosophila melanogaster* (fruit fly, Rom and Kahana, 1993), *Danio rerio* (zebrafish, Hascilowicz *et al.*, 2002), and *Xenopus laevis* (African clawed frog, Bassez *et al.*, 1990; Cao *et al.*, 2001).

However, the knowledge of the molecular structure of ODC in the marine fishes is extremely limited. In addition, the nature of ODCs in these fish and their roles in the control of polyamine pathway are still unclear. The flounder (*Paralichthys olivaceus*) is a commercially important marine aquaculture species in Korea, and it has been used for molecular-level studies of various functional genes (Cho *et al.*, 2001; Lee *et al.*, 2001; Kim and Kim, 1999).

In this study, The isolation of cDNA encoding ODC and the expression of ODC in adult tissues are focused on. These data will provide a wider base of knowledge on the primary structure of ODC at the molecular level and its functional diversity.

EXPERIMENTAL PROCEDURES

MATERIALS

Bacterial strains

The *E.coli* XL1-Blue MRF'[F'::*Tn10 proA*$^{+}$*B*$^{+}$ *lac Iq Δ(lacZ) M15/recA1 endA1 gyrA96(Nalr) thi hsdR17(rk mk*$^{+}$*) supE44 relA1 lac*] was used for transformation and color selection. BL21 (DE3) codon plus [F *ompT hsd*S(r_B m_B) *dcm gal* Tet^{r} λ(DE3) *end*A Hte (*argU ileY leuW Cam*^{r})] was used for the overexpression of flounder ODC gene.

Tissues

The Brain, Liver, Kidney, Embryo were obtained from mature Flounders (n=10; size: 45±10㎝, body weight: 900±300 g; 3 years old). and frozen at -70℃ until use.

Enzymes

Restriction enzymes such as Xho I , EcoR I , Pst I , Kpn I , Xba I and T4 ligase were purchased from Promega Inc. (U.S.A.). Taq

polymerase and Reverse Transcriptase were obtained from Bioneer (Taejeon, Korea). Sequencing ready reaction kit (ABI PRISM^{TM} dye terminator) was purchased from Perkin Elmer (U.S.A.).

Plasmid DNA and Oligonucleotide primers

Wizard plus SV minipreps DNA purification system was purchased from Promega Inc. (U.S.A.) for plasmid DNA purification. The oligonucleotide primers used for this study were summarized in Table 1. The primers designed for ODC cloning based on the conserved sequences from known ODC sequences were synthesized from GenoTech Inc. (Taejeon, Korea). Primers for ODC-RF, ODC-RR, ODC-exF, ODC-ns were also synthesized from the sequences obtained from the ODC clone. The location of these primers were shown in Figure 2.

Other Materials

pGEM-T Easy vector (Promega Inc.) was used for ligation of the PCR fragments. pET101/D-TOPO expression vector (Invitrogen) was used for expression of *E.coli* system. DIG labeling and detection kit were purchased from Boehringer Mannheim.

Table 1. Oligonucleotide Primers used for this study

primers	sequences	Remarks
ODC-F	5'-GTCAACAT(T/C)AT(T/C)GCCAA(G/A)AAGGTC'-3	Forward primer for preparation of probe
ODC-R	5'-CC(G/C)ACGGTGTAGGC(G/A)CCCATG-3'	Reverse primer for preparation of probe
ODC-RF	5'-CCACCGATTTTGAATTCCC-3'	Forward primer for RT-PCR
ODC-RR	5'-CTCAAATTTGAGGAGATCACAG-3'	Reverse primer for RT-PCR
ODC-exF	5'-CACCATGAACACTGCAACTC-3'	Forward primer for expression
ODC-ns	5'-CTCGAGAACCACACGGG-3'	Reverse primer for expression

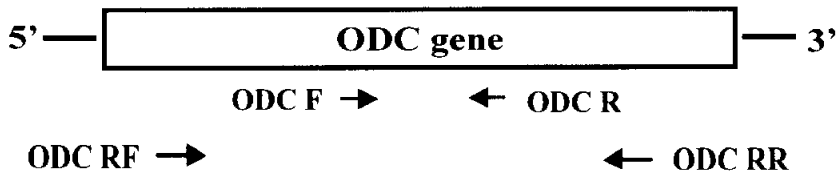

Figure 2. Location of primers used for cloning of ODC gene

Nitro blue tetrazolium chloride (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) for alkaline phosphatase color developing solutions were purchased from Bio-Rad. Protein low molecular weight marker and Prestained protein molecular weight marker were obtained from Fermentas. TRIzol reagent for total RNA isolation was purchased from Invitrogen. Other chemicals were purchased from Fluka and Sigma (U.S.A.).

METHODS

Total RNA isolation from flounder

Total RNA from flounder brain, liver and kidney tissues were isolated using a TRIzol reagent (Invitrogen). To extract total RNA, tissues were homogenized in 1 ml of TRIzol reagent per 100 mg of tissue using homogenizer (PYREX). The sample was kept on ice 5 min and 200 $\mu\ell$ of chloroform was added and vigorous shaked for 15 sec. Then, the sample kept on room temperature and centrifuged for 20 min at 12,000 rpm. The aqueous phase was carefully transferred to a new tube, and 0.5 volume of isopropanol was added and mixed by inverting. the sample was centrifuged for 15 min at 12,000 rpm and supernatant was discarded. The pellet was washed with 300 $\mu\ell$ of 75% ethanol and dried briefly. The pellet was dissolved in DEPC-treated water. The quality was assessed by formaldehyde RNA gel electrophoresis.

cDNA library construction and Screening of ODC cDNA

Total RNA was isolated using a Micro-FastTrack™ 2.0 Kit (Invitrogen). A flounder brain cDNA library was constructed using a ZAP-cDNA Synthesis Kit (Stratagene). The resulting library contained approximately 1×10^5 clones. The library was then amplified up to 3×10^9 clones/ml. The Conserved nucleotide sequences of vertebrate ODCs were determined using National Center for Biotechnology Information (NCBI) nucleotide and protein database, and oligonucleotide degeneracy primers for probe preparation for screening ODC were synthesized at GenoTech (Taejeon, Korea). The probe used to screen for ODC was amplified by PCR. The main PCR program consisted of 30 cycles at 94℃ for 30 sec, 52℃ for 30 sec, and 72℃ for 30 sec. Probe for screening ODC was labeled with a DIG (digoxigenin) oligonucleotide 3'-end labeling kit (Roche). DIG labeled probe was quantified with standard immunoscreening procedure and detection system. Approximately, 1×10^5 plaques from the cDNA library were screened using this probe. Positive plaques recovered from the first screening were confirmed with a second screening. Positive plaques were recovered from the second screening and the phagemid containing the insert was excised according to the manufacturer's instructions (Stratagene). The excised phagemid was performed by DNA sequencing.

Reverse transcription polymerase chain reaction (RT-PCR)

In order to perform RT-PCR, total RNA was isolated from brain, muscle, liver and embryo from mature flounder. The RT-PCR was performed using Bioneer's RT-PCR system. The reaction components were set up for Master mix 1 and master mix 2. Master mix 1 contained temple RNA, 50 pmol oligo dT primer, DEPC-water. The sample was incubated for 10 min at 65℃ and cooled down on ice. Master mix 2 consisted of 5× RT-PCR buffer, 2.5 mM dNTP mixture, 100 mM DTT, RNase inhibitor, and M-MLV RTase. Mix 1 and mix 2 were added to a 0.2 $\mu\ell$ tube. The sample was placed in a thermocycler GeneAmp PCR system 2400 (Perkin Elmer) and incubated for 1 hr at 42℃ for reverse transcription followed by thermocycling. The temperature profile consisted of an initial 94℃ for 5 min and 30 cycles at 94℃ 40 sec, 54℃ for 30 sec, and 72℃ for 1 min, and a final 7 min extension at 72℃. After reaction, Fifteen microliters of the reaction products were analyzed with 1% agarose gel electrophoresis. The PCR product was eluted from the agarose gel and cloned into pGEM Easy T-vector system (Promega) (figure 3). *E.coli* XL1-blue strain was transformed with the ligated DNA.

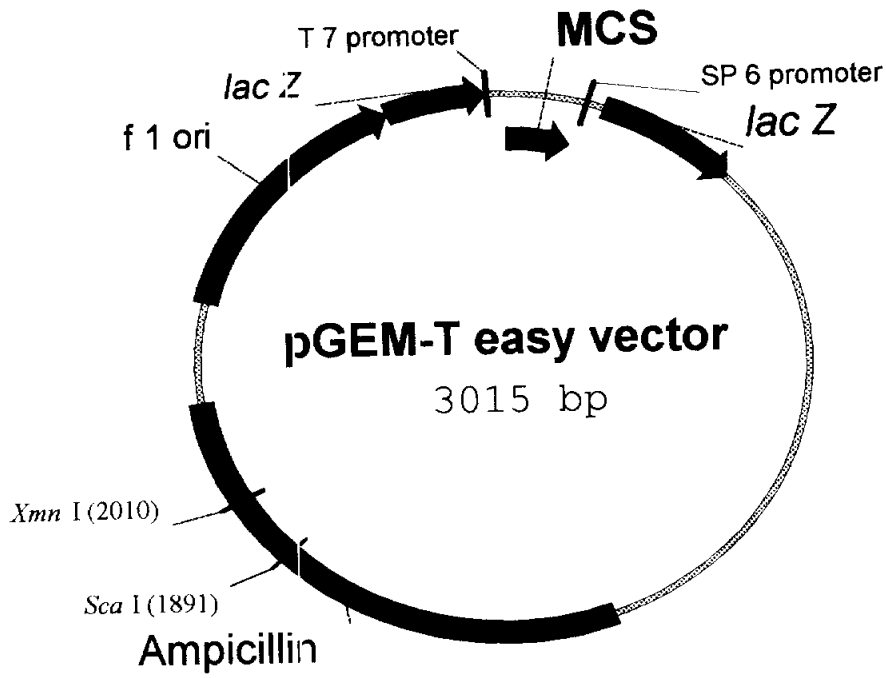

Figure 3. pGEM-T Easy Vector Map.

It contains a T7 and SP6 RNA polymerase transcription initiation site and multiple cloning site (MCS). and also offers sequencing primer sites. The high copy number pGEM-T Easy vector contain T7 and SP6 RNA polymerase promoters flaking a multiple cloning region within the α -peptide coding region of the enzyme β-galactosidase.

DNA purification and Sequencing

Cloned plasmid DNA was purified using Wizard plus SV minipreps DNA purification system (promega). Sequencing reaction was performed using ABI PRISM^{TM} DNA sequencing kit (Perkin Elmer). Sequencing reaction mixture (500 ng of template DNA, 1 pmol of primer, 4 $\mu\ell$ of terminator ready reaction mixture, and distilled water to 20 $\mu\ell$ volume) was prepared and placed into a thermal cycler. Thermal cycling with 25 cycles of program (96℃ 10 sec denaturation, 50℃ 6 sec annealing, 60℃ 4 min extension) was performed. The products were precipitated with ethanol and dissolved in 25 $\mu\ell$ of Template Suppressing Reagent (Perkin Elmer). The sample was denatured for 2 min at 95℃, cooled down on ice and analyzed using ABI 310 genetic analyzer (Perkin Elmer).

Expression of ODC gene in *E.coli*

In order to express the ODC gene, the cloned ODC cDNA was subcloned into pET101/D-Topo expression vector (Invitrogen), which allows expression of a recombinant protein with C-terminal fusion His-tag. In order to insert ODC gene into pET101/D-Topo, The ODC gene was amplified by PCR using a pair of the

'ODC-exF' and 'ODC-ns' (Table. 1) and eluted. The resulting plasmid that inserted the ODC gene was transformed into *E.coli* strain BL21(DE3) codon plus. The cells harboring the expression plasmid that contained the ODC gene were cultured overnight at 37℃ in a LB medium (containing 50 ㎍/ml ampicillin).

The cells were induced by adding IPTG (Isopropyl-β -D-thiogalactopyranoside) to a final concentration of 1 mM at cell density corresponding to OD_{600}= 0.5~1.0. After induction, the cells were harvested by centrifugation at 0, 1, 3 hours, respectively.

Analysis of expressed protein on SDS-PAGE

The induced cell was centrifuged and the pellet was mixed with 2×sample loading buffer (0.5 M Tris-HCl pH 6.8, Glycerol, 10% SDS, β-Mercaptoethanol, distilled water, 0.05% (w/v) bromophenol blue) and heated at 100℃ for 3 min. The sample were centrifuged 13,000 rpm for 2 min and stored on ice. Twenty microliters of each samples were loaded on a 12% SDS-polyacrylamide gel (12% seperating gel, pH 8.8 overlayed 4% stacking gel, pH 6.8). The samples were electrophoresed under constant current of 20 mA for 2 hr. The gel was stained with coomassie brilliant blue and destained with destaining buffer (7% acetic acid, 15% methanol).

Western blot analysis

After total proteins containing ODC-His protein were seperated on a 10% SDS-acrylamide gel electrophoresis, the gel was transferred to nitrocellulose membrane at 30 mA 16 hr in transfer buffer (25 mM Tris, 120 mM glycine, 20% methanol). The nitrocellulose membrane was washed with TTBS buffer (20 mM Tris-Cl, pH 7.4, 0.5 M NaCl, 2.5 mM KCl, 0.1% Tween-20) and blocked with TBS buffer containing 5% skim milk for 1 hr. The membrane was washed three times for 5 min with TTBS buffer and treated for 1.5 hr with His-tag primary antibody which diluted 1:10,000 in TTBS containing 5% skim milk. After washing three times for 5 min, the membrane was treated for 1 hr with alkaline phosphatase conjugated anti-goat IgG in TTBS containing 5% skim milk. The membrane was washed three times for 5 min in TTBS buffer and developed by NBT and BCIP solution in alkaline phosphate buffer (0.1 M Tris-Cl, pH 9.5, 0.1 M NaCl, 50 mM $MgCl_2$).

The purification of ODC-His fusion protein by affinity chromatography.

The ODC-His tag fusion protein eluted using His Trap Kit (Amersham pharmacia biotech). The cultured cells were harvested by centrifugation. The cell pellets were resuspended with resuspension buffer (20 mM Tris-HCl, pH 8.0) and sonicated three times for 10 sec with Ultra sonicator, and harvested again by centrifugation at 13,000 rpm for 10 min. The supernatant was discarded. The cell pellets were resuspended by adding isolation buffer (2 M Urea, 20 mM Tris-HCl, 0.5 M NaCl, 2% Triton X-100, pH 8.0) and sonicated. The supernatant of centrifugated cell was discarded. The cell pellets were resuspended in binding buffer (6 M guanidine hydrochloride, 20 mM Tris-HCl, 0.5 M NaCl, 5 mM imidazole, 1 mM 2-mercaptoethanol, pH 8.0). The resuspended cell debris was discarded and the supernatant was obtained. The sample was filterated with 0.45㎛ filter. The prepared sample was eluted by affinity purification using HiTrap Chelating HP column.

Comparative sequence analysis of flounder ODC

To examine the molecular evolution of ODC, the following vertebrate ODC sequences were imported from the SwissProt databank/GenBank: *H. sapiens* (NP-002530), *B. taurus* (AAA79849), *M. musculus* (NP-038642), *R. norvegicus* (NP-03647), *M. pahari* (AAA39847), *C. griseus* (DCHYOC), *X.*

laevis (P27120 for ODC 1; Q9I8S4 for ODC 2), *D. rerio* (BAB84694), and *P. olivaceus* (AY214169). The nucleotide sequences were analyzed using the program BLAST (http://www.ncbi.nlm.nih.gov/BLAST). A multiple sequence alignment was conducted using the program CLUSTAL W (http://www.ebi.ac.uk/clustalw) and sequence identities were calculated using GeneDoc (http://www.psc.edu/biomed/genedoc). A phylogenetic tree was constructed by the neighbor-joining(NJ) method using the program Treecon (Van de Peer and De Wachter, 1994) for the amino acid sequences of ODCs from *H. sapiens*, *B. taurus*, *R. norvegicus*, *M. musculus*, *C. griseus*, *X. laevis* (ODC 1 and ODC 2), *D. rerio*, and *P. olivaceus*.

RESULTS

Preparation of the ODC probe and Screening

In order to identify the ODC gene from flounder, degenerate oligonucleotide primers encoding parts of the conserved domains were prepared and used to produce a probe using a flounder brain cDNA library, constructed using a λ ZAP-II cDNA Synthesis Kit. As shown in Figure 4, the deduced nucleotide sequence of the ODC cDNA is approximately 300-bp. The PCR product was sequenced by ABI automatic sequence analyzer (Perkin Elmer) and confirmed as the flounder ODC cDNA fragment. Probe for screening ODC was labeled with a DIG (Digoxigenin) oligonucleotide 3' end labeling Kit (Roche). Concentration of probe was relatively determined with comparison to a Dig-labeled control-DNA in a dot blot followed by direct immunodetection with the color substrate NBT/BCIP. The concentration of Dig-labeled ODC probe DNA is approximately 5×10 ng/$\mu\ell$. Approximately, 1×10^5 plaques were screened. Several positive clones were obtained and analyzed their nucleotide sequences.

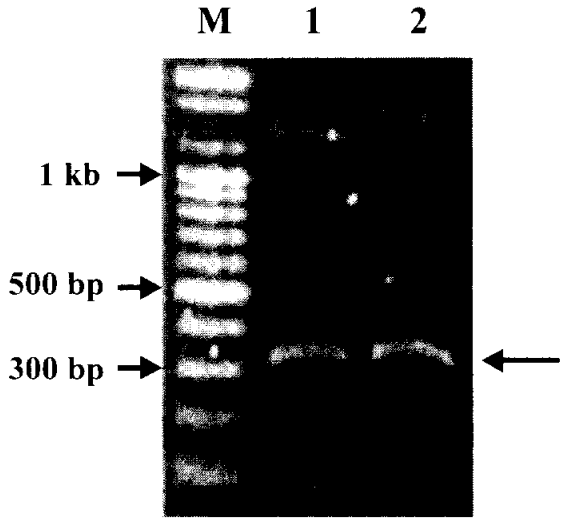

Figure 4. PCR amplification for ODC probe preparation

An arrow indicate PCR product. The expected DNA fragment band (about 300 bp) was detected. land M; a molecular weight marker. lane 1 and 2; the PCR patterns according to template concentrations.

Determination of ODC gene expression at the transcription level using RT-PCR

To investigate the tissue distribution of the flounder ODC gene, a reverse transcription-polymerase chain reaction (RT-PCR) was performed with primers specific for the flounder ODC using total RNA isolated from flounder tissues as a template. Approximately, 730 bp DNA fragment was amplified by RT-PCR. This DNA fragment was analyzed with cloning and sequencing. As shown in Figure 5, the expression of the ODC gene was detected in embryo, brain, young brain, kidney, and liver.

The characterization of ODC cDNA

The nucleotide sequence of the complete cDNA encoding the flounder ODC gene and its deduced amino acid sequence is shown in Figure 6. DNA sequence of cloned ODC cDNA was aligned with NCBI (National Center for Biotechnology Information) DNA sequence date base by Blast program. The sequence contains a 312 bp 5'-untranslated region (UTR) followed by a 1380 bp coding region, which corresponds to a 460 amino-acid protein, and a 1247 bp 3'-UTR. As shown in Figure 6, the flounder ODC cDNA clone contains an in-frame

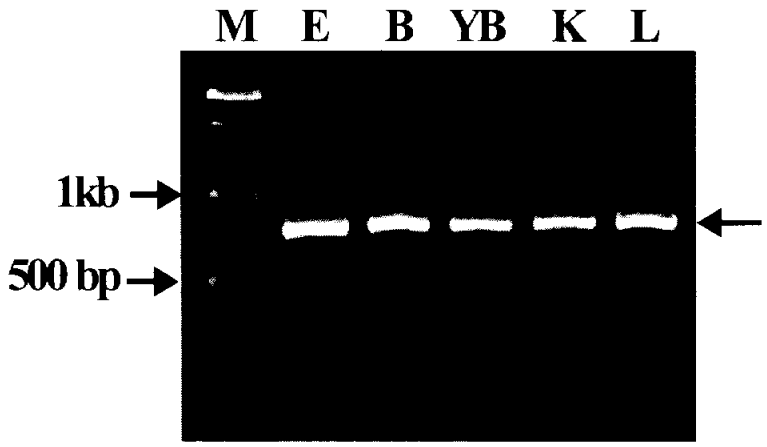

Figure 5. Patterns of the expression of ODC detected by RT-PCR.

Lane "M" indicates a molecular marker. "E" indicates Embryo; "B" (Brain); "YB" (Young Brain); "K" (Kidney); "L" (Liver).

An arrow indicate ODC RT-PCR product. The expected DNA fragment band (about 730 bp) was detected.

```
   1  GCACGAGCTCGTGCCGAATTCGGCACGAGAAGCCACACCTCCAGCAGCGGGCTCCATTCAGAGGGACAACTGCTTCTTTTTAATCCCGGC
  91  GGTCGTGTCTAATGGACAAACTCTTTGTAAATGCTTTTTAAGAACTTTAACTCTAGGAGATCACACACTGGCTACTCAACAGGCCCTGGC
                M  D  K  K  F  V  N  A  F  *
 181  CTTGAGTCTTATCTGAAGGGGTTTAACTTCACTGGTGAGCCACCTTGGCTTCTCTGCACCTGAGGAACAGCAGCACTTAATTCCATCTCT
 271  TTTCAATTTACTTTTGAGTAAACAAAAGGACTTGTTGTTACCATGAACACTGCAACTCCCACCGATTTTGAATTCCCTTTCCTGGAGGAG
                                                M  N  T  A  T  P  T  D  F  E  F  P  F  L  E  E    16
 361  GGTTTCACTGCCCGTGATGTTGTTGAGCAGAAGATCAATGAATCATCTATGACGGATGATAGAGATGCCTTCTATGTCTGCGACTTGGGG
       G  F  T  A  R  D  V  V  E  Q  K  I  N  E  S  S  M  T  D  D  R  D  A  F  Y  V  C  D  L  G    46
 451  GATGTTCTTAAGAAACACCTGCGCTGGATGAGGGCCCTGCCTCGCATCACTCCTTTCTATGCTGTCAAGTGCAATGACAGTCGGGCAGTC
       D  V  L  K  K  H  K  R  W  M  R  A  L  P  R  I  T  P  F  Y  A  V  K  C  N  D  S  R  A  V    76
 541  GTTACAACACTGGCATCCCTAGGCACTGGATTTGACTGTGCGAGCAAGACGGAGATCCAGCTGGTTCAGTCTCTGGGAGTGGATCCAAGC
       V  T  T  L  A  S  L  G  T  G  F  D  C  A  S  K  T  E  I  Q  L  V  Q  S  L  G  V  D  P  S   106
 631  AGAATCATCTATGCCAACCCCTGCAAGCAAGTTTCGCAGATCAAGTATGCGTCTGCCCATGGGGTCCAGATGATGACCTTTGATAGTGAA
       R  I  I  Y  A  N  P  C  K  Q  V  S  Q  I  K  Y  A  S  A  H  G  V  Q  M  M  T  F  D  S  E   136
 721  GTGGAACTCATGAAAGTGGCCCGTTGTCATGACAATGCCAAGCTGGTGCTGCGTATCGCCACAGATGACTCAAAGGCAGTGTGTCGTCTG
       V  E  L  M  K  V  A  R  C  H  D  N  A  K  L  V  L  R  I  A  T  D  D  S  K  A  V  C  R  L   166
 811  AGTGTGAAGTTTGGGGCCCCGCTCAAAGCTTGTCGAGGTCTTCTGGAGCGGGCTAAAGAACTGGGACTGAACGTGATCGGTGTCAGCTTC
       S  V  K  F  G  A  P  L  K  A  C  R  G  L  L  E  R  A  K  E  L  G  L  N  V  I  G  V  S  F   196
 901  CATGTTGGCAGTGGCTGTACTGATTCCAAGACCTACATGCAGGCCATCGCTGATGCTCGCTGTGTTTTCCATATGGGGGATGAGCTCGGC
       H  V  G  S  G  C  T  D  S  K  T  Y  M  Q  A  I  A  D  A  R  C  V  F  H  M  G  D  E  L  G   226
 991  TTCAACATGGATCTCTTGGACATTGGCGGTGGTTTCCCTGGTTCAGACAATGTTGAACTCAAATTTGAGGAGATCACAGCTGTAATCAAC
       F  N  M  D  L  L  D  I  G  G  G  F  P  G  S  D  N  V  E  L  K  F  E  E  I  T  A  V  I  N   256
1081  TCTGCCCTGGACAAGTATTTCTCTGCTGACTCTGGTGTTAAGATCATTGCTGAGCCAGGACGCTTTTATGTAGCTTCAGCTTACACACTA
       S  A  L  D  K  Y  F  S  A  D  S  G  V  K  I  I  A  E  P  G  R  F  Y  V  A  S  A  Y  T  L   286
1171  GTTGTCAACATTATTGCCAAGAAGGTCATCATGGACGAGGACTCAGTCTCTGACGATGAAGATGAAGGGACCAATGACAGGACTCTGATG
       V  V  N  I  I  A  K  K  V  I  M  D  E  D  S  V  S  D  D  E  D  E  G  T  N  D  R  T  L  M   316
1261  TACTATGTCAATGATGGAGTGTACGGATCTTTCAACTGTATACTCTATGACCACGCTCATTGTATGCCAATACTGCACAAGAAACCAAAG
       Y  Y  V  N  D  G  V  Y  G  S  F  N  C  I  L  Y  D  H  A  H  C  M  P  I  L  H  K  K  P  K   346
1351  CCAGATGAGATCATGTACCCCTGCAGCATCTGGGGCCCAACTTGTGATGGTCTTGATCGCATTGTTGAGCAGTGTTACTTGCCTGACATG
       P  D  E  I  M  Y  P  C  S  I  W  G  P  T  C  D  G  L  D  R  I  V  E  Q  C  Y  L  P  D  M   376
1441  CAGGTGGGCGACTGGCTGGTCTTTGAAAACATGGGTGCCTACACTGTGGCTGCCTCTTCCACCTTCAATGGTTTCCAAAGACCTGACCTT
       Q  V  G  D  W  L  V  F  E  N  M  G  A  Y  T  V  A  A  S  S  T  F  N  G  F  Q  R  P  D  L   406
1531  CACTATGTCATTTCCCGTCCTGCTTGGCAACACGTGCAGCAGATTTGTTTGCAGGGCATGCCAGCCCCTGTGGAGGAGTCTCGCTTGTTC
       H  Y  V  I  S  R  P  A  W  Q  H  V  Q  Q  I  C  L  Q  G  M  P  A  P  V  E  E  S  R  L  F   436
1621  GAAGTGTCTGCATGCTGCGGCCAAGAGAGCAGCTTGGAGATGCCTACCAAGCCTTGCCAGGCCCGTGTGGTTTAAACCGAGACATTTCTA
       E  V  S  A  C  C  G  Q  E  S  S  L  E  M  P  T  K  P  C  Q  A  R  V  V  *                  460
1711  CATATCTAGCATCCTTTGTTTTCAAATATTGGAGAAGATTCTATTGGGTTTTTTCCCCCTGCTTTCTCCCCAAATGTATGTGAACATTTA
1801  AGGCCAGTTACTTGACAGGAGAATGAGAGGGGTATGTTTTGCACAAAATTTCTCTGTTCTGTATGGAAGCTGGAGATCAACCTTCAATGA
1891  AATGAGGCTAAATCACACTCTGAATGTCTCTATGAGTGTAACAAGGCAACGCTGCACTTGAATGTGTTAGTGCAGCCTTCTTGCCCTAGT
1981  CAGGGGAAATGACTGTGGGAATTAGATCTTGAAGAATCTGTTTGCTGAATTGATAGTCAATTGCTAAATACATCACAAAACCACTTTGAT
2071  GAAGGTTCCTCATCACATTGGTCTTTACATTTGGACAAACTCTTTTTGATTGTCCTAGAGGTGAAGAGTTACTAATGCTATTCACATATG
2161  TTAAAGAATAATTGCAAATTGCTTATCAGCTGAAGTCACATCCCTTGCTAGTAACTCACAAAGAGGTGATTAGAGGGGTGATTTAATTCC
2251  TTAAATGGCTGCTATGTTATTATCTTTTCCATGATCTCCACTTTTGTACAGAACTCCAAGTGTTAATCCTCAAGATGTTAATGCATAAAG
2341  AAACGCCATATTATTATTTTAGTCTCATTCTATATTTCATTAAGTATTTGATGTGTGTTCTTTAAATACTTTCCTGCTGCTCATTGCCAG
2431  AATTACTCCAAATGCTAAATTTAAATGTGTACACTCACAGGAAATACCTGTAAATATGTGCATAAATCCTGTTCTGTATGTAAAAACACC
2521  ACATGTCGCAACTGGGCCAATACAGCTGCAGCAAATGAGACAACTTGTCACACAACAATATAGATACTTACAGTTCATTGACATCTCAGC
2611  TACCCATGATCCTCATGTTTGTCCTGCTTAGTGGACACAACATGAATCTTTAAGATTAACACTTAAAACACAGGGACTCAAGAAGCAGAC
2701  TGCACCCCTTCTCTTCTCTTCCTTATATTATTCTCTTTTTATGTTTTGTAACTTTTAAAAGGCAAGAAATCTATTTGTGATATTAATAAC
2791  ATGTTTAACTACACATATGACATAGACACAAAAGAGATGGGATTGCGATTTCATTGAGCTTTCCTGTGGAAACGATTAGAAGTTTTGTAT
2881  TTTTTTAATAAATAAAATGTGTGTTTGGTGCAACTACGAAAAAAAAAAAAAAAAAAAAA
```

Figure 6. The nucleotide and deduced amino acid sequences of cDNA encoding flounder ODC.

The nucleotide numbers are shown on left side and amino acid numbers are shown on right side. An *asterisk* indicates the stop codon. The consensus polyadenylation signal and internal ribosome entry site is underlined.

termination codon (TAA) at base 1693-1695. A putative polyadenylation signal (AATAAA) can be found 26 bp upstream from the poly(A) tail. Both the 5' and 3' UTRs of the mammalian ODC are reported to play critical roles in translational regulation of the ODC gene. The 5'-UTR is considered responsible for hampering ODC mRNA expression, while the 3'-UTR interferes with this translation-suppressing effect and participates in the 5'-UTR-independent hypotonic ODC induction. As Figure 6 shows, the 5'-UTR of the flounder ODC gene contains nucleotides predicted to form a short upstream open reading frame (MDKKFVNAF, spanning nucleotides 102-129), and a potential internal ribosome entry site. This sequence might lead to different mechanisms of translational regulation in flounder ODC. An alignment of the amino acid sequence of the flounder and other ODCs is shown in Figure 7. The deduced flounder amino acid sequence was about 80.3% and 70.6% identical with zebrafish and human ODC, respectively. Also, It has a high similarity in amino acid residues with the other species, greater than 60% sequence identity. A molecular phylogenetic tree was constructed to analyze the evolutionary relationships of the ODC proteins (Figure 8). It shows the evolutionary divergence of the ODC genes of zebrafish, flounder, frog, mouse, rat, Chinese hamster, cow, and human.

```
H. sapiens      MNNFGNEEFDCHFLDEGFTAKDILDQKINEVSSSDDKDAFYVADLGDILKKHLRWLKALP 60
B. taurus       MNSFSNEEFDCHFLDEGFTAKDILDQKINEVSYSDDKDAFYVADLGDILKKHLRWLKALP 60
M. musculus     MSSFTKDEFDCHILDEGFTAKDILDQKINEVSSSDDKDAFYVADLGDILKKHLRWLKALP 60
M. pahari       MSSFTKEEFDCHILDEGFTAKDILDQKINEVSSSDDKDAFYVADLGDILKKHLRWLKALP 60
R. norvegicus   MGSFTKEEFDCHILDEGFTAKDILDQKINEVSSSDDKDAFYVADLGDVLKKHLRWLKALP 60
C. griseus      MNSFNKDEFDCHILDEGFTAKDILDQKINEVSS-DDKDAFYVADLGDVLKKHLRWLKALP 59
X. laevis 1     MNSFSNDDFDFSFLEEGFSARDIVEQKINEVSLSDDKDAFYVADFGDIVKKHVRWFKALP 60
X. laevis 2     MQGYIQ-ESDFNLVEEGFLARDLMEEIINEVSQTEDRDAFFVADLGDVVRKHLRFLKALP 59
D. rerio        MTACTGSDFDFAFLEEGFCARDIVEQKINESSLSDDKDAFYVADLGDVLKKHLRWLRVLP 60
P. olivaceus    MNTATPTDFEFPFLEEGFTARDVVEQKINESSMTDDRDAFYVCDLGDVLKKHLRWMRALP 60
                *      : :  :::*** *:*:::: *** *  :*:***:*.*:**:::**:*:::.**

H. sapiens      RVTPFYAVKCNDSKAIVKTLAATGTGFDCASKTEIQLVQSLGVPPERIIYANPCKQVSQI 120
B. taurus       RVTPFYAVKCNDSRTIVKTLAAIGTGFDCASKTEIQLVQSLGVPPERIIYANPCKQVSQI 120
M. musculus     RVTPFYAVKCNDSRAIVSTLAAIGTGFDCASKTEIQLVQGLGVPAERVIYANPCKQVSQI 120
M. pahari       RVTPFYAVKCNDSRAIVSTLAATGTGFDCASKTEIQLVQGLGVPPERIIYANPCKQVSQI 120
R. norvegicus   RVTPFYAVKCNDSRAIVSTLAAIGTGFDCASKTEIQLVQGLGVPPERIIYANPCKQVSQI 120
C. griseus      -VTPFYAVKCNDSRALVNTLAAITV--DCASKTEIQLVQGLGVPPERVIYANPCKQVSQI 116
X. laevis 1     RVTPFYAVKCNDGKAIVKTLSILGAGFDCASKTEIQLVQSIGVSPERIIYANPCKQVSQI 120
X. laevis 2     RVKPFYAVKCNSSKGVVKILAELGAGFDCASKTEIELVQDVGVAPERIIYANPCKQISQI 119
D. rerio        RITPFYAVKCNDSRAVVTTLASLGAGFDCASKTEIQIVQSVGVDPSRIIYANPCKQVSQI 120
P. olivaceus    RITPFYAVKCNDSRAVVTTLASLGTGFDCASKTEIQLVQSLGVDPSRIIYANPCKQVSQI 120
                 :.********..: :*. *:   .  ********::**.:** .*:********:***
```

Figure 7. Multiple alignment of the predicted flounder ODC amino acid sequence with known ODC protein sequences taken from GenBank; The accession numbers are given in the text. Amino acid residues that are identical in the ODC proteins are marked with an *asterisk*.

```
H. sapiens      LLERAKELNIDVVGVSFHVGSGCTDPETFVQAISDARCVFDMGAEVGFSMYLLDIGGGFP 239
B. taurus       LLERAKELDIDVIGVSFHVGSGCTDPETFVQAISDARCVFDMGAEVGFNMYLLDIGGGFP 239
M. musculus     LLERAKELNIDVIGVSFHVGSGCTDPDTFVQAVSDARCVFDMATEVGFSMHLLDIGGGFP 239
M. pahari       LLERAKELNIDVIGVSFHVGSGCTDPETFVQAVSDARCVFDMGTEVGFSMYLLDIGGGFP 239
R. norvegicus   LLERAKELNIDVIGVSFHVGSGCTDPETFVQAVSDARCVFDMGTEVGFSMYLLDIGGGFP 239
C. griseus      LLERAKELNIDVIGVSFHVGSGCTDPETFVQALSDARCVFDMGTEVGFSMYLLDIGGGFP 236
X. laevis 1     LLERAKELNVDIIGVSFHVGSGCTDPQTYVQAVSDARCVFDMGAELGFNMHLLDIGGGFP 239
X. laevis 2     LLEMAKNLSVDVIGVSFHVGSGCTDSKAYTQAISDARLVFEMASEFGYKMWLLDIGGGFP 238
D. rerio        LLERAKELGLDVIGVSFHVGSGCTDPETYSQAISDARYVFDIGAELGYNMSLLDIGGGFP 239
P. olivaceus    LLERAKELGLNVIGVSFHVGSGCTDSKTYMQAIADARCVFHMGDELGFNMDLLDIGGGFP 239
                *** **:*.::::************..:: **::*** **.:. *.*:.* *********

H. sapiens      GSEDVKLKFEEITGVINPALDKYFPSDSGVRIIAEPGRYYVASAFTLAVNIIAKKIVLKE 299
B. taurus       GSEDVKLKFEEITSVINPALDKYFPSDSGVRIIAEPGRYYVASAFTLAVNIIAKKLVLKE 299
M. musculus     GSEDTKLKFEEITSVINPALDKYFPSDSGVRIIAEPGRYYVASAFTLAVNIIAKKTVWKE 299
M. pahari       GSEDTKLKFEEITSVINPALDKYFPSDSGVRIIAEPGRYYVASAFTLAVNIIAKKTVWKE 299
R. norvegicus   GSEDTKLKFEEITSVINPALDKYFPSDSGVRIIAEPGRYYVASAFTLAVNIIAKKTVWKE 299
C. griseus      GSEDTKLKFEEITSVINPALDKYFPPDSGVRVIAEPGRYYVASAFTLAVNIIAKKIVSK- 295
X. laevis 1     GSEDVKLKFEEITSVINPALDKYFPADSGVKIIAEPGRYYVASSFTLAVNIIAKKVMVNE 299
X. laevis 2     GTEDSKIRFEEIAGVINPALDMYFPESSDVQIIAEPGRYYVASAFSLAVNVIAKKEVEH- 297
D. rerio        GSEDTKLKFEEIAAVINPALDKYFPVDSGVRIIAEPGRYYVASAYTLAVNIIAKKVIMKE 299
P. olivaceus    GSDNVELKFEEITAVINSALDKYFSADSGVKIIAEPGRFYVASAYTLVVNIIAKKVIMDE 299
                *::: :::****:.***.*** **. .*.*::******:****:::*.**:**** : .

H. sapiens      QTGSDDE-DESSEQTFMYYVNDGVYGSFNCILYDHAHVKPLLQKRPKPDEKYYSSSIWGP 358
B. taurus       QTGSDDE-EESTDRTFMYYVNDGVYGSFNCILYDHAHVKPLLQKRPKPDEKYYSSSIWGP 358
M. musculus     QPGSDDE-DESNEQTFMYYVNDGVYGSFNCILYDHAHVKALLQKRPKPDEKYYSSSIWGP 358
M. pahari       QPGSDDE-DESNEQTFMYYVNDGVYGSFNCILYDHAHVKALLQKRPKPDEKYYSSSIWGP 358
R. norvegicus   QTGSDDE-DESNEQTLMYYVNDGVYGSFNCILYDHAHVKALLQKRPKPDEKYYSSSIWGP 358
C. griseus      --GSDDE-DESSEQTFMYYVNDGVYGSFNCILYDHAHVKPLLPKRPKPDEKYYSSSIWGP 352
X. laevis 1     QSGSDDEEDAANDKTLMYYVNDGVYGSFNCILFDHAHVKPVLTKKPKPDEKFYSSSIWGP 359
X. laevis 2     -SVSDDE-ENESSKSIMYYVNDGVYGSFNCLVFDHAHPKPILHKKPSPDQPLYTSSLWGP 355
D. rertio       QSASDEEEDVSNDRTLMYYVNDGVYGSFNCILYDHAHVLPTLHKKPKPDERMYPCSIWGP 359
P. olivaceus    DSVSDDEDEGTNDRTLMYYVNDGVYGSFNCILYDHAHCMPILHKKPKPDEIMYPCSIWGP 359
                  **:* :  ..:::***************:::****  . * *:*.**:: *..*:***
```

Figure 7. —continued

```
H. sapiens        TCDGLDRIVERCDLPEMHVGDWMLFENMGAYTVAAASTFNGFQRPTIYYVMSGPAWQLMQ 418
B. taurus         TCDGLDRIVERCNLPEMHVGDWMLFENMGAYTVAAASTFNGFQRPTIYYVMSGPTWQLMQ 418
M. musculus       TCDGLDRIVERCNLPEMHVGDWMLFENMGAYTVAAASTFNGFQRPNIYYVMSRPMWQLMK 418
M. pahari         TCDGLDRIVERCNLPEMHVGDWMLFENMGAYTVAAASTFNGFQRPNIYYVMSRPMWQLMK 418
R. norvegicus     TCDGLDRIVERCSLPEMHVGDWMLFENMGAYTVAAASTFNGFQRPNIYYVMSRSMWQLMK 418
C. griseus        TCDGLDRIVERCNLPEMHVGDWMLFENMGAYTVAAASTFNGFQRPSIYYVMSRPMWQLMK 412
X. laevis 1       TCDGLDRIVERFELPELQVGDWMLFENMGAYTVAAASTFNGFQRPTLYYVMSRPHWQLMH 419
X. laevis 2       TCDGLDQIAERVQLPELHVGDWLLFENMGAYTIAASSNFNGFQQSPVHYAMPRAAWKAVQ 415
D. rerio          TCDGLDRIVEQCSLPDMQVGDWLLFENMGAYTVAASSTFNGFQKPDIYYIMSRTAWQCMQ 419
P. olivaceus      TCDGLDRIVEQCYLPDMQVGDWLVFENMGAYTVAASSTFNGFQRPDLHYVISRPAWQHVQ 419
                  ******:*.*:  **:::****::********:**:*.*****:. ::* :. . *: ::

H. sapiens        QFQNPDFPP-EVEEQDASTLPVSCAWESGMKRHRAACASASINV 461
B. taurus         QIRTQDFPP-GVEEPDVGPLPVSCAWESGMKRHSAACASTRINV 461
M. musculus       QIQSHGFPP-EVEEQDDGTLPMSCAQESGMDRHPAACASARINV 461
M. pahari         RIQSHGFPP-EVEEQDDGTLPMSCAQESGMDHHSAACASARINV 461
R. norvegicus     QIQSHGFPP-EVEEQDVGTLPMSCAQESGMDRHPAACASASINV 461
C. griseus        QIQNHGFPP-EVEEQDVGTLPISCAQESGMDRHPAACASASINV 455
X. laevis 1       DIKEHGILP-EVP--DLSALHVSCAQESGMELAPAVCTAASINV 460
X. laevis 2       LLQR-GLQQ--TEEKENVCTPMSCGWEISDSLCFTRTFAATSII 456
D. rerio          QIRAQGIPALPLEEPSAGNVPSHCGRESSLDVPAKPCPTQVL-- 461
P. olivaceus      QICLQGMPA-PVEESRLFEVSACCGQESSLEMPTKPCQARVV-- 460
                    :   .:               *. * . .       :
```

Figure 7. —continued

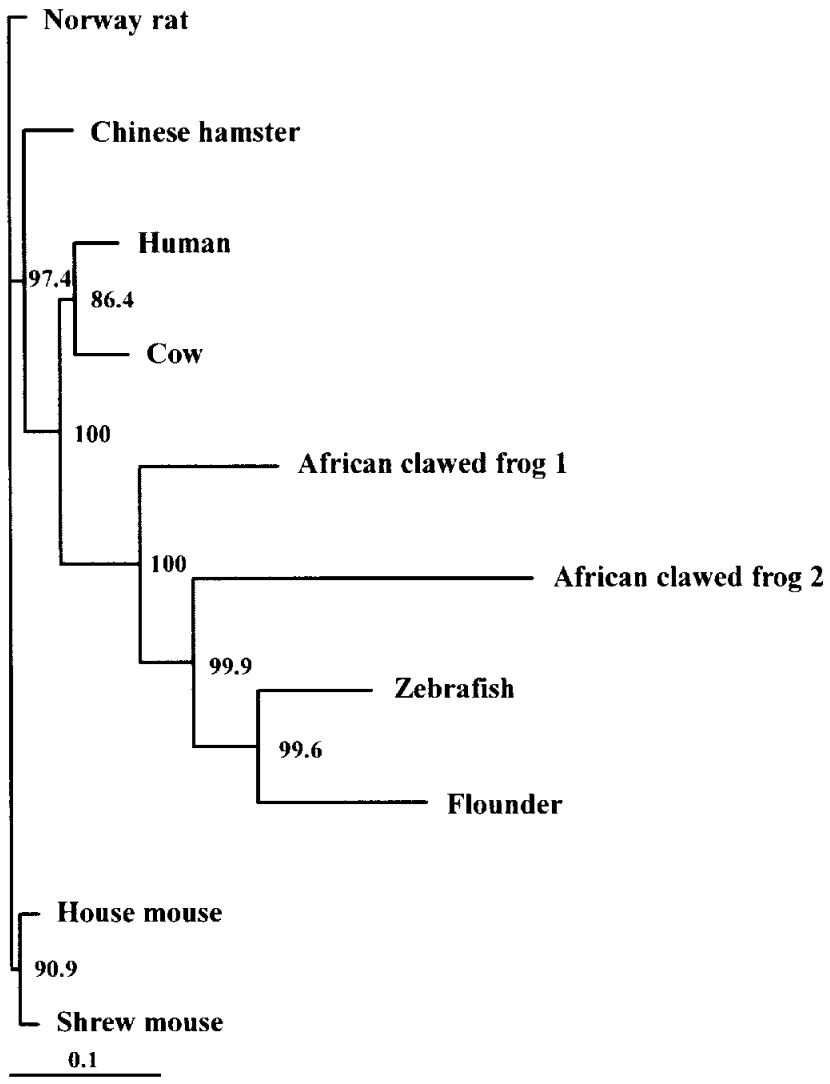

Figure 8. A molecular phylogenetic tree of ODC based on the NJ method.

The values shown on each internal branch are the percentage support determined from a bootstrap analysis with 1,000 replications.

The flounder ODC protein was more closely related to the zebrafish ODC than to the human ODC as reflected in the sequence identity (80.3 vs. 70.6%).

The highest level of similarity was in residues responsible for active site formation and stabilization of the ODC, which are almost identical in the species dimers. Many structural elements are involved in regulating ODC translation and degrading the enzyme (antizyme-binding element of ODC protein and its C-terminal end). The flounder ODC contains residues that are important for formation of the ODC dimer (D134, K169, K294, Y324, Y332, D365, G388, F398), the enzymes active site (K69, D88, R154, K169, H197, G235, G236, G237, R277, Y324, D333, C361, D362, Y390, N399), the putative antizyme binding region (residues 117-140) and two degradation elements (corresponding to residues 376-424 and 422-461 of the human and mouse ODC sequences) in the C terminus. The flounder ODC also contains N-linked glycosylation site (N-X-S/T) (residues 29-31; 71-73).

Overexpression of flounder ODC gene in *E. coli*

To express the flounder ODC gene in a prokaryotic system, the ODC cDNA was subcloned into pET101/D-TOPO expression vector. The resulting pET101/D-TOPO-ODC plasmid (Figure 9) was transformed into *E.coli* BL 21(DE3) codon plus.

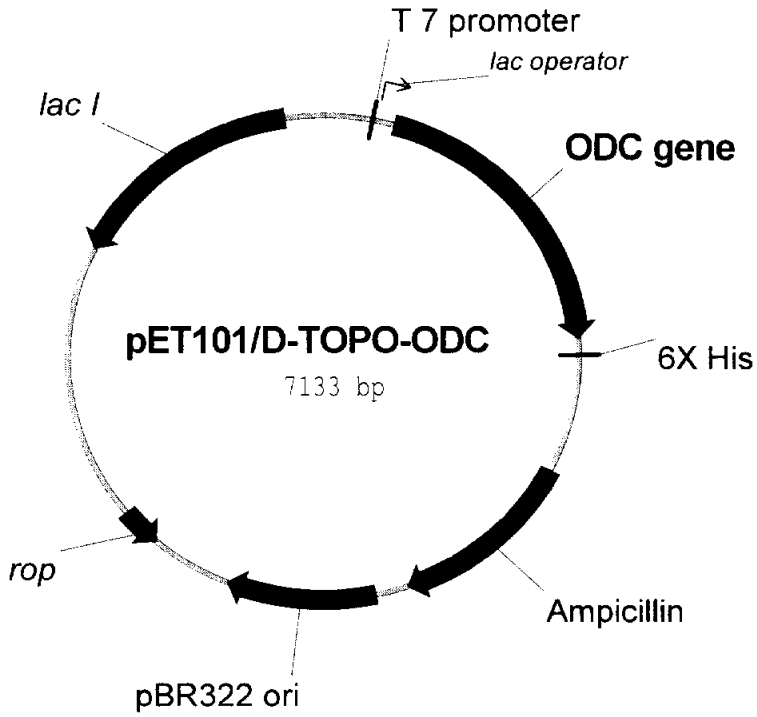

Figure 9. Schematic map of recombinant pET101/D-TOPO vector for ODC expression

It contains a T7 lac promoter for chemically inducible, high level expression and allows expression of recombinant protein with a native C-terminal fusion tag. In order to subclone the ODC gene into pET101/D-TOPO vector, the reverse PCR primer was designed to remove the native stop codon in the gene of the interest.

The expression patterns of the ODC proteins were analyzed using SDS-PAGE and shown in Figure 10A. The cloned ODC gene was strongly expressed after IPTG induction. The optimum induction time was approximately 3 hr after IPTG induction. As shown in Figure 10A, the molecular weight of ODC-His fusion protein is approximately 53 kDa, while the predicted actual protein size of ODC is approximately 50 kDa. Because the pET 101/D-TOPO expression vector has a C-terminal fusion tag (3 kDa).

Purification of ODC protein and Western blot analysis

The expressed protein was purified by affinity chromatography. The result of purified ODC protein is shown in lane 5 of Figure 10A. The purified ODC protein was determined as a molecular weight of 53 kDa single band. In order to perform Western blot, proteins were electrophoretically transferred from an SDS-PAGE gel to a nitrocellulose membrane, probed with goat antiserum against 6-His tag, and incubated with alkaline phosphatase coupled to goat antibody against goat IgG. The nitrocellulose membrane developed using NBT/BCIP. As shown in Figure 10B, Western blot was analyzed and confirmed.

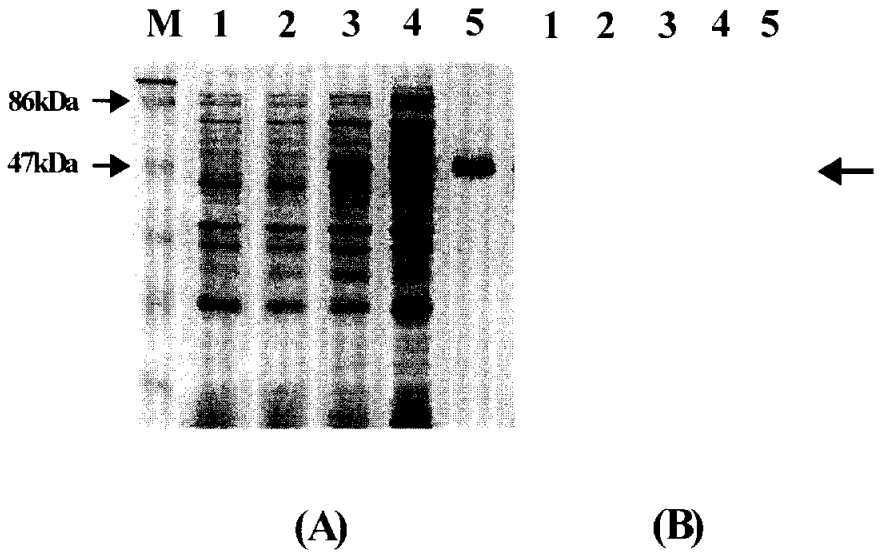

Figure 10. Analysis of the expressed proteins using SDS-PAGE and Western blotting.

(A) The expressed proteins were analyzed using 12% SDS-PAGE. Lane M, standard protein molecular weight markers; lane 1, proteins from uninduced cell extracts (control); lanes 2-4, proteins from induced cell extracts 0, 1, and 3 hr after IPTG induction, respectively; lane 5, purified ODC protein.

(B) Western blot analysis of expressed proteins.

Lanes 1-5, proteins used the same as (A).

DISCUSSION

Fish are the largest and most diverse group of vertebrates. Their evolutionary position relative to other vertebrates, and their ability to adapt to a wide variety of environments make them ideal for studying both organism and molecular evolution. ODC is a key enzyme in the biosynthesis of polyamines, as it regulates intracellular concentration of polyamine, which are essential for cell growth and function. Several ODC genes have been studied in fish, and some have been analyzed at the molecular level.

This study provides phylogenic information on ODC that is essential for understanding the molecular evolution of this gene in vertebrates. It may be necessary to conduct a comparative analysis of the structure, expression, and function of the ODC gene in order to elucidate the mechanism responsible for controlling the biosynthetic polyamine pathway and the intracellular polyamine concentrations. Fish are the most primitive vertebrates, and genetic information obtained from fish can reveal the origin and diversion of genes with a similar function in other organisms. Observations and genetic manipulations of the flounder ODC make this species a very useful model for studying the mechanism of polyamine participation in the ODC regulation system during development.

In conclusion, the flounder ODC cDNA consisted of 2,939 bp

encoding 272 amino acid residues and has a 5'-noncoding region (312 nucleotides) and a 3'- noncoding region (1247 nucleotide). The flounder ODC have a short ORF in a 5' UTR region, suggesting that it might play different mechanisms of translational ODC regulation in flounder. The 5' and 3' UTRs of mammalian ODC are important in translational regulation of the enzyme, though the details of this regulation are not fully understood. The cloned ODC cDNA showed 80.3% and 70.8% amino acid sequence identity to that of zebrafish and rat, respectively. A comparison of the amino acid sequences of vertebrate ODC indicated that the flounder ODC is highly conserved with those of other species. The resulting RT-PCR DNA banding patterns provided evidence for the expression of ODC in tissues from the embryo, brain, young brain, kidney, and liver. The flounder ODC mRNA has a wide tissue distribution. The recombinant flounder ODC was efficiently expressed in *E.coli*. Therefore, this results will contribute to a deeper understanding of the mechanisms involved in the polyamine-mediated ODC regulation system.

국 문 초 록

Ornithine decarboxylase (ODC)는 폴리아민 생합성 과정에 관여하는 중요한 조절효소이다. 폴리아민은 세포 증식 및 분열을 촉진하고, embryo의 성숙을 도우며, 핵산의 구조형성 및 복제, 단백질 합성 등의 여러 가지 세포의 대사과정에 중요하게 관여하고 있다. 또한 ODC는 비타민 B_6 활성형인 pyridoxal 5′ phosphate (PLP)를 cofactor로 요구하는 homodimeric 효소이다.

넙치의 ornithine decarboxylase을 암호화하는 cDNA 클론을 넙치로부터 분리하여 클로닝하고 sequencing을 통하여 분석하였다. 얻어진 ODC cDNA 염기서열에 대한 유전적 유사성 및 상관성 분석을 NCBI(National Center for Biotechnology Information) Data base을 이용하여 조사한 결과, ODC cDNA는 zebrafish의 ODC cDNA와 80.3%, 쥐의 ODC cDNA와 70.8%가 유사함을 알 수 있었고, 또한 아미노산 서열을 비교한 결과 동일의 결과를 얻을 수 있었다. 넙치의 ODC cDNA는 1380 염기의 ORF (Open reading frame)와 460 아미노산을 가지고 있으며 분자량은 50kDa 이었다. 이러한 넙치의 ODC cDNA의 구조와 염기서열을 zebrafish와 쥐의 구조와 염기서열과 비교했을 때 매우 높은 유사성을 가지고 있었다. 넙치의 각 조직별로 ODC 특이적 발현 여부를 알아보기 위해 reverse- transcription chain reaction을 수행하였다. 그 결과 ODC mRNA는 넙치의 Brain, Kidney, Liver, 그리고 Embryo에서 발현되었다. Histidine을 tagging하고 있는 recombinant 넙치 ODC 단백질을 유전자 재조합법에 의해 구축한 후에, 대장균에서 대량발현을 유도하였다. 이렇게 발현한 ODC 단백질을 affinity 크로마토그레피 방법을 이용하여 정제하였으며, 분리된 단백질을 Western blot으로 확인하였다.

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