Available online at www.sciencedirect.com
ScienceDirect
Marine Pollution Bulletin 57 (2008) 433–440
MARINE POLLUTION BULLETIN
www.elsevier.com/locate/marpolbul

Molecular cloning and β-naphthoflavone-induced expression of a cytochrome P450 1A (*CYP1A*) gene from an anadromous river pufferfish, *Takifugu obscurus*

Jin-Hyoung Kim [a], Sheikh Raisuddin [b], Jang-Seu Ki [b], Jae-Seong Lee [b,*], Kyung-Nam Han [a,*]

[a] *Department of Marine Science, College of Natural Sciences, Inha University, Incheon 402-751, South Korea*
[b] *Department of Chemistry, and the National Research Lab of Marine Molecular and Environmental Bioscience, College of Natural Sciences, Hanyang University, Seoul 133-791, South Korea*

Abstract

In recent years, there has been a decline in the wild populations of river pufferfish, *Takifugu obscurus.* Besides overexploitation for commercial purposes, environmental pollution is believed to have contributed to its decline. However, almost no information exists about genes involved in metabolism of xenobiotics by this species. Nevertheless, there is interest in fugu fishes, since they possess the smallest genome among vertebrates. We cloned and characterized the full-length cDNA sequence of a cytochrome P450 1A (*CYP1A*) gene from *T. obscurus*. Phylogenic relationship of *T. obscurus CYP1A* was also compared to other fish species. The tissue distribution and time-dependant induction of *CYP1A* mRNA were studied by real-time PCR in *T. obscurus* exposed to an aryl hydrocarbon receptor (Ahr) agonist, β-naphthoflavone (BNF). The greatest basal expression in livers of control as well as BNF-treated individuals. However, brain, gill, gonad, intestine, and kidney also expressed *CYP1A*. Muscles expressed the least *CYP1A*. The results of the time-course study revealed induction in brain and gills after 6 h and at 12 h in most tissues. Except for gills, all other organs retained induced expression of *CYP1A* mRNA up to 96 h.

Keywords: Population decline; Tissue distribution; Xenobiotic metabolism

1. Introduction

The full genome sequences of two pufferfish, *Takifugu rubripes* and *Tetraodon nigroviridis* have generated great interest due to their genomics. The genomes of both of these fish species are among the shortest in vertebrates (Aparicio et al., 2002; Jaillon et al., 2004). The genomics of *Takifugu obscurus* (river puffer) have been less studied. A recent study shows that wild populations of this species are declining because of overexploitation and possibly due to environmental pollution (Yang and Chen, 2004). However, to our knowledge, toxicity ranges and information on metabolism of xenobiotics are not available in this fish species. Additionally, *T. obscurus* is an anadromous and euryhaline fish that must cope with varying salinity during its life cycle. It has been suggested that *T. obscurus* is a suitable model fish species for study of osmoregulation (Kato et al., 2005). These features make it an interesting organism for study of stress responses and metabolism of xenobiotics. In Korea *T. obscurus* is highly valued for its nutritive properties, and aquaculture of pufferfish has been initiated recently in Korea. Other countries, particularly China and Japan also have commercial interests in this fish. It lives in the bottom layer of inshore and inland waters and most of the growth takes place in the sea but spawning in freshwater (Kato et al., 2005). In principle, *T. obscurus* experiences the environmental changes of both freshwater and saltwater habitats and accordingly aquatic pollutants of a diverse

* Corresponding authors. Tel.: +82 2 2220 0769; fax: +82 2 2299 9450.
E-mail addresses: jslee2@hanyang.ac.kr (J.-S. Lee), knhan@inha.ac.kr (K.-N. Han).

0025-326X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.marpolbul.2008.01.005

kind. Therefore, it was deemed interesting to study activation of xenobiotics and detoxification genes including cytochrome P450 (*CYP*) in this fish.

CYP genes play an important role in the metabolism of xenobiotics and steroid metabolism (Williams et al., 1998; Hu et al., 2004; Goldstone and Stegeman, 2006; Isin and Guengerich, 2007). Habitats of *T. obscurus* may be contaminated by environmental pollutants. Therefore, information of xenobiotic metabolizing genes from this fish is desirable to understand its responses to exposure to xenobiotics directly or indirectly. Recently, we characterized a metallothionein (*MT*) gene from *T. obscurus* (Kim et al., in press-b). Here we report on the molecular characterization of *CYP1A* gene and its expression profile in *T. obscurus* exposed to a universal inducer of *CYP1A*, β-naphthoflavone (BNF). We selected *CYP1A* because it is the major gene involved in xenobiotic metabolism in fish and has been used as a biomarker of exposure (Bucheli and Font, 1995; Arinc et al., 2000; Rees and Li, 2004; Hu et al., 2007).

2. Materials and methods

2.1. Fish

T. obscurus (body length, 14.5 ± 1.4 cm; body mass, 40.5 ± 4.7 g) were procured from Yang-chon Fish Hatchery (Gimpo, Kyounggi-do, Korea) and transported to the laboratory avoiding any physical stress. They were acclimatized to laboratory conditions for 2 wk at 24 °C and a photoperiod of 12 h light–12 h dark in an 100-l tank containing well-aerated water (pH 7.80, dissolve oxygen, DO 5.6 mg/l). During the acclimation period, fish were fed a commercial diet S7 (Higashimaru Foods, Inc., Kagoshima, Japan).

2.2. RNA isolation, reverse transcription, first strand cDNA synthesis

Total RNA from the liver of fish was isolated by homogenizing the tissue with Trizol® reagent (Molecular Research Center, Inc., Cincinnati, OH, USA). The first strand cDNA was made as a template for real-time reverse transcriptase-polymerase chain reaction (RT-PCR), ™ III reverse transcriptase (Invitrogen, Carlsbad, CA, USA) according to the manufacturers' protocol.

2.3. PCR, cloning, amplification of 3′ and 5′ ends

The degenerative primers for *CYP1A* were designed using the conserved domains after multiple alignments of cDNA sequences of teleosts *CYP1A* available in NCBI database. Primer and PCR conditions are provided elsewhere (Table 1). The PCR product was eluted from the gel using a specially designed kit (Quiagen Inc., Valencia, CA, USA) and subcloned into pCR2.1 TA vector using chemically competent bacterial cells (Invitrogen, Carlsbad, CA, USA). The plasmid DNA was extracted from the bacterial culture using the kit (Promega, Madison, WI, USA). Sequence analysis was performed using ABI PRISM 3700 DNA sequencer at Bionics (Seoul, Korea). The full-length sequence of *T. obscurus CYP1A* was deduced by the 3′-Rapid Amplification of cDNA Ends (3′-RACE) and 5′-RACE using GeneRacer kit (Invitrogen, Carlsbad, CA, USA).

2.4. Phylogenetic position of T. obscurus inferred from CYP1A gene

To determine phylogenetic relationship for the *CYP1A* gene, complete CYP1A amino acid sequence determined here and some of those obtained from the DDBJ/EMBL/GenBank were aligned using the Clustal X 1.83 (Thompson

Table 1
Primer and PCR condition details

Gene	Oligo name	Sequences (5′ → 3′)	Nucleotide position	Remarks	PCR condition
CYP1A	RT-F	GCATGCTGGAGGAGCACATTTGC	485–507	cDNA amplification	95 °C/5 min: 40 cycle of 98 °C/25 s, 50 °C/40 s, 72 °C/90 s: 72 °C/10 min
	RT-R	CTCCGATGCAGCGGCGCTTCC	1376–1396		
	3GSP1	CTGAAGAGCAACGTGGATCAGAATCG	1054–1079	3′-RACE	94 °C/3 min: 35 cycle of 98 °C/25 s, 55 °C/60 s, 72 °C/90 s: 72 °C/10 min
	3GSP2	GCAGATCAACCACGATCCTGAGC	1251–1396		
	5GSP1	AGAGAGTCCGTAATGTC	853–869	5′-RACE	94 °C/2 min: 35 cycle of 94 °C/60 s, 55 °C/60 s, 72 °C/2 min: 72 °C/7 min
	5GSP2	GGTTACCGCTGCTCACCACTTTGC	689–712		
	5GSP3	CAGAGACGACGATGTGAGGGAAAGG	571–595		
	CYP RT-F	AAGGTCATGATCTTCGGCTTGG	1324–1345	real-time PCR	94 °C/5 min: 35 cycle of 94 °C/30 s, 50 °C/30 s, 72 °C/30 s: 72 °C/7 min
	CYP RT-R	GCGCTTGTGCTTCATGGTGAGG	1452–1473		
β-actin	RT-F	CATCACCATCGGCAACGAGAGG	780–801		
	RT-R	CGTCGCACTTCATGATGCTGTTG	881–904		