Molecular characterization and organs expression of cytochrome P450 1B1 from Japanese eel (*Anguilla japonica*)

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ABSTRACT

The CYP1 family, one of the gene families of the CYP superfamily, has four subfamilies deposited in the GenBank/EMBL so far; CYP1A, CYP1B, CYP1C, and the newly identified CYP1D. The metabolic activation and elimination of polyaromatic hydrocarbons, polychlorinated biphenyls, and aryl amines from fish body is largely mediated by the CYP enzymes. A new cDNA of the CYP1B subfamily encoding CYP1B1 was isolated from Japanese eel liver after a single intraperitoneal injection of β-naphthoflavone (BNF). The full-length cDNA obtained was 2985 bp and contained a 5’ noncoding region of 294 bp, an open reading frame of 1626 bp coding for 541 amino acids and a stop codon and a 3’ noncoding region of 1065 bp. The predicted molecular weight of the protein was approximately 61.27 kDa. The deduced amino acid sequence of Japanese eel CYP1B1 showed 62% similarity to three-spined stickleback CYP1B1 and zebrafish CYP1B1. It exhibited similarities of 66% with that of killifish, Indian medaka and our previously reported carp CYP1B1 and -1B2 while the higher similarities (67 and 69%) of the deduced amino acids was observed with that of Nile tilapia CYP1B1 and rainbow trout respectively. The percent identities of Japanese eel CYP1B1 cDNA showed similarities with those of the reported CYP1Bs of mammals of 57, 57, and 56% for human, rat, and mouse CYP1B1, respectively. Japanese eel CYP1B1 was aligned with the CYP1 sequences and has been deposited in the GenBank/EMBL data bank with the accession number AY518340. The phylogenetic tree constructed using the previously reported CYP1B sequences of mammals and fish suggested the closer relationship of the newly identified Japanese eel CYP1B1 to rainbow trout CYP1B1. QRT-PCR analysis of liver, kidney, gills and intestine revealed a distinct induced expression in liver, kidney and gills (71.93, 3.87 and 539.56 respectively) while the constitutive expression (0.062) was observed in intestine.

Keywords: *Anguilla japonica*, β-naphthoflavone, cytochrome P450, CYP1B1.

INTRODUCTION

In aquatic animals that inhabit environments often contaminated with high levels of organic pollutants, the induction of CYP genes as well as the catalytic activity of their products can serve as a sensitive biomarker for environmental contamination by dioxin and other chemical compounds (Payne 1976; Goksoyr 1995; Monostory *et al.*, 1996). Cytochrome P450 enzymes are intermediates to the metabolic activation of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and aryl amines. The cytochrome P450 enzymes catalyze the metabolic reactions of a variety of substrates from endogenous compounds such as steroids, fatty acids, vitamins, prostaglandins, and retinoids to numerous exogenous chemicals such as drugs,
carcinogens, mutagens, and other chemical pollutants often resulting from combustion process (Nebert & Gonzalez, 1987; Guengerich, 1990; Porter & Coon, 1991; Coon et al., 1996; Nelson et al., 1996). Compared with humans that possess three CYP1 genes named CYP1A1, CYP1A2 and CYP1B1 (Nebert & Russell, 2002), fish such as medaka, zebrafish and killifish possess five genes: CYP1A, CYP1B1, CYP1C1, CYP1C2 and CYP1D1 (Goldstone et al., 2007, 2009). Until the recent identification and characterization of the CYP1B1 gene in mammals (Savas et al., 1994, Bhattacharyya et al., 1995, Walker et al., 1995, Tang et al., 1996) and fish (Leaver & George, 2000, Godard et al., 2000, El-Kady et al., 2004a,b, Willett et al., 2006, Hou-Chu et al., 2008, Zanette et al., 2009; 2010), CYP1A1 and CYP1A2 were the main CYP1 family members held responsible for the oxidation and activation of PAHs, PCBs and aryl amines. Some laboratory chemicals like β-naphthoflavone (BNF) and 3-methylcholanthrene (3-MC) are regarded as prototypical PAHs-type inducers of CYP genes. These chemicals exert their effects on mammalian CYP1A and CYP1B through the interaction of the ligand-bound aromatic hydrocarbon receptor (AHR) and its specific binding sequences located upstream of the respective target genes (Hakinson, 1995; Zhang et al., 1998; Buters et al., 1999). The isolated five CYP1s from fish present a markedly distinct organ-specific distribution in the gene expression levels suggesting that different physiological roles could exist (Jönsson et al., 2007b; Goldstone et al., 2009). Up to date, only a single isoform of CYP1B has been identified from all fish species, whereas both CYP1B1 and CYP1B2 genes have been cloned in carp (Cyprinus carpio).

Recent studies on metabolism and carcinogenesis have shown mammalian CYP1B1 to be a critical and in some cases, necessary enzyme in the activation of several pollutants, notably the PAH 7,12-dimethylbenzene[a]anthrathene (DMBA) (Buters et al., 1999; Chun and Kim, 2003). They reported that CYP1B1 is located exclusively at extrahepatic sites and mediates the carcinogenesis of DMBA. Also, Shimada et al., 1996; 2002) reported that CYP1B1 participates with CYP1A1 and CYP1A2 in the activation of 2,3,7,8-tetrachlorodibenzo-p-dioxin, benzo[a]pyrene and related carcinogens causing initiation of cancers in human and mice. CYP1B1 is also potentially playing a role in endocrine regulation and estrogen-induced carcinogenesis (Hayes et al., 1996). Human CYP1B1 has been shown to metabolize 17 β-estradiol to a 4-hydroxylated product, a chemical considered to cause breast cancer in women (Spink et al., 1998).

In contrast to CYP1As which have been identified in most vertebrate groups (mammals, birds, fish), CYP1Bs have been identified in a few mammal and fish species. The Genbank survey on fish CYP1Bs revealed only nine sequences; our previously reported carp CYP1B1 and -1B2, Nile tilapia sequence from our laboratory, and CYP1B1 sequences of plaice, catfish, Indian medaka, killifish, rainbow trout and zebrafish.

In this study, a cDNA of the CYP1B1 gene was isolated from the liver of BNF-treated Japanese eel and sequenced. Phylogenetic analysis was also performed to assess the relationship of this newly identified CYP1B1 gene with the other CYP1B subfamily members. Also the expression pattern of CYP1B1 mRNA was determined in liver, kidney, intestine and gills using QRT-PCR.
MATERIALS AND METHODS

Fish treatment and RNA preparation

Japanese eel (Anguilla japonica) with a mean weight of 400 g were obtained from a commercial source and were treated with a single intraperitoneal injection of BNF (100 mg/kg) suspended in corn oil. Similar control fish were intraperitoneally injected at the same time with an equivalent volume of the vehicle (corn oil). Three days under fasting conditions, the treated and the control fish were killed and samples of the liver, kidney, intestine and gills were collected, immediately frozen in liquid nitrogen, and stored at –80 °C. Total RNA was isolated from 2 g of each of the samples of frozen liver, kidney, intestine and gills according to the Standard Acid Guanidinium Thiocyanate Phenol Choloroform (AGPC) extraction method (Chomczynski & Sacchi, 1978). Total RNA concentration and purity were determined spectrophotometrically as described by Sambrook & Russel (2001), and A260/A280 ratios were between 1.7 and 1.9. Poly (A)+ RNA was purified using an Oligotex-dt30 mRNA purification kit (Takara, Japan).

Reverse transcription for full-length cDNA generation

Reverse transcription of mRNA was performed with Superscript II reverse transcriptase (Gibco BRL, USA) to generate 5'-RACE-Ready and 3'-RACE-Ready first-strand cDNAs using the SMART™ RACE (rapid amplification of cDNA ends) cDNA amplification kit (Clontech, USA). According to the manufacturer’s protocol, 3’- and 5’-RACE Ready cDNA to be used as a template in the 3’ and 5’ RACE PCR respectively.

3’ and 5’-RACE PCRs for full-length cDNA

We designed one sense (F) and an antisense (R) primer specific to Japanese eel CYP1B1 for 3’ and 5’ RACE PCRs, respectively. The primer sequences were designed from short sequence obtained at our laboratory and is given in Table 1. The sense and antisense gene specific primers were used in combination with the universal primer mix (UPM) of the RACE PCR kit to generate the RACE PCR products. The RACE PCR reactions with the reverse transcription (RT) products obtained were conducted according to the principles of touch down PCR in order to avoid spurious amplifications (Don et al., 1991).

Table 1: Oligonucleotide primers used in the PCR amplification of Japanese eel CYP1B1 cDNA fragments.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Nucleotide sequence</th>
<th>Nucleotide location</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>5’-GTTTATCGATGCAACGCTCAGGC</td>
<td>2111 to 2133</td>
</tr>
<tr>
<td>R</td>
<td>5’-GCCGATCACAGGTCATGCTTAGTA</td>
<td>597 to 574</td>
</tr>
<tr>
<td>UPM (long)</td>
<td>5’-CTAATACGACTCACTATAGGGCAAGCAGTGGTGTTAGCAGG</td>
<td>2111 to 2133</td>
</tr>
<tr>
<td>UPM (short)</td>
<td>5’-CTAATACGACTCACTATAGGGCC</td>
<td>597 to 574</td>
</tr>
</tbody>
</table>

The cycle conditions for RACE PCR were as follows: 5 cycles of denaturation for 5 s at 94°C and annealing for 3 min at 72 °C; 5 cycles of 94 °C for 5 s, 70 °C for 10 s, and 72 °C for 3 min; and 35 cycles of 94 °C for 5 s, 68 °C for 10 s, and 72 °C for 3 min. The DNA band of expected size was excised with a scalpel, purified using a GFX PCR DNA and a gel band purification kit (Amersham Biosciences, USA). The PCR products were subjected to restriction mapping with various enzymes and were cloned into PT7BlueT-vector (Novagen, USA). Purified plasmids were directly sequenced by dye terminator cycle sequencing using the ABI PRISM dye terminator cycle sequencing kit (PE Biosystemes, USA) and an Applied Biosystems 3100 DNA Sequencer.
Phylogenetic analysis

DNA sequences with the following GenBank accession numbers were retrieved from the database and used in the phylogenetic analysis: DQ088663 (channel catfish CYP1B1), FJ786959 (killifish CYP1B1), HQ202282 (three-spined stickleback fish CYP1B1), NM_001174149 (rainbow trout CYP1B1), JX4546651 (Indian medaka CYP1B1), AK728664 (zebrafish CYP1B1), AK437774 (carp CYP1B1), AK437775 (carp CYP1B2), HQ829968 (Nile tilapia CYP1B1), U03688 (human CYP1B1), U09540 (rat CYP1B1) and X78445 (mouse CYP1B1). In order to determine homology among CYP1B subfamily cDNAs or deduced amino acid sequences of various species, sequence alignments were performed by the CLUSTAL W multiple sequence alignment method (online alignment site constructed by Kyoto University, Japan, http://www.genome.jp/tools/clustalw/). The phylogenetic tree was constructed by unweighted pair group method (UPGM) using the amino acid sequences of the previously reported CYP1B subfamily sequences of teleosts and mammals.

CYP1B1 expression in different organs of Japanese eel using QRT-PCR Reverse transcription

Reverse transcription of the RNA samples isolated from liver, kidney, intestine and gills was performed using Primescript™ RT reagent kit (Takara, Japan) according to the manufacturer’s instructions. Reactions were incubated for 15 min at 37°C then 5 s at 85°C to inactivate the reverse transcriptase. RT products were stored at 4°C for further PCRs.

Primer design for QRT-PCR reaction

Primers for Japanese eel CYP1B1 cDNA were designed using Laser gene primer select program (Ver. 5.52, 2003, DNASTAR Inc), with melting temperatures (Tm) ranging from 58 to 60°C, and amplicon lengths of 50 to 150 bp. The primer sequences are given in Table (2).

<table>
<thead>
<tr>
<th>Primer</th>
<th>Nucleotide sequence</th>
<th>Nucleotide location</th>
<th>Product length</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>5'- GTTTATCGATGCAACGCTCAGGC</td>
<td>2111 to 2133</td>
<td>92bp</td>
</tr>
<tr>
<td>R</td>
<td>5'- TGAGGACAACAACAACAACAACACTA</td>
<td>783 to 806</td>
<td></td>
</tr>
</tbody>
</table>

Quantitative Real-time PCR conditions and analysis

Each PCR reaction consisted of 10 μl of SYBR® Premix Ex Taq™ II (2X), 10 μM of each primer, 2 μl of cDNA template and double distilled water to a final volume of 20 μl. All standard plasmid DNA dilutions, template controls and induced samples were run in triplicates. Reactions were then analyzed on an ABI 7300 Real-Time PCR system under the following conditions: 35 cycles of 94°C for 30 s, 58°C for 30 s and 72°C for 3 min. The relative quantification relates the PCR signal of the target transcript in a treatment group to that of another sample such as an untreated control (calibrator). For quantification of induced Japanese eel CYP1B1 normalized to an untreated control, standard curves were prepared for both the induced and the untreated reference. Each of the normalized induced Japanese eel CYP1B1 values was divided by the untreated control normalized value to generate the relative expression levels. Accordingly, CYP1B1 mRNA levels were reported as fold change in abundance relative to the average calibrator response.

Statistical analysis

The statistical differences between the groups were determined, and the data expressed as mean ± standard deviation. Excel (Microsoft, NY) were used to analyze the data, and Student’s t test was used for the comparisons. A P-value <0.05 was considered significant. At least three determinations were carried out for each data point.
RESULTS

Nucleotide sequence analysis

Figure 1 shows the full-length cDNA sequence (2985 bp) of Japanese eel cytochrome P450, CYP1B1. The obtained nucleotide sequence of Japanese eel CYP1B1 contained a 5' noncoding region of 294 bp, an open reading frame (ORF) of 1626 bp coding for 541 amino acids and a stop codon, and a 3' noncoding region of 1065 bp. The predicted molecular weight of the protein was approximately 61.27 kDa. The sequence has one polyadenylation signals (AATAAA) and a poly A tail of 30 nucleotides. This sequence was aligned with the CYP1 sequences and has been deposited in the GenBank/EMBL data bank with the accession number AY518340.

Comparison of amino acid sequences

The open reading frame and its deduced amino acid residues of Japanese eel cytochrome P450, CYP1B1 cDNA is shown in Figure 2. Table 3 shows the similarity percentage of the deduced amino acid sequences of Japanese eel CYP1B1 with those of the previously reported other CYP1B1 members. The deduced amino acid sequence of Japanese eel CYP1B1 showed 62% similarity to three-spined stickleback CYP1B1 and zebrafish CYP1B1. It exhibited similarities of 66% with that of killifish, Indian medaka and our previously reported carp CYP1B1 and -1B2 while the higher similarities (67 and 69%) of the deduced amino acids was observed with that of Nile tilapia CYP1B1 and rainbow trout respectively. The percent identities of Japanese eel CYP1B1 cDNA showed similarities with those of the reported CYP1Bs of mammals of 57, 57, and 56% for human, rat, and mouse CYP1B1, respectively.

Fig. 1: Nucleotide sequence (2985 bp) of Japanese eel cytochrome P450 CYP1B1 cDNA. Consensus sequence for polyadenylation signal is bolded.

Fig. 2: The open reading frame (ORF) and its deduced amino acid residues of Japanese eel cytochrome P450 CYP1B1 cDNA.
Table 3: Percent identities of deduced amino acid sequences of CYP1B subfamily genes.

<table>
<thead>
<tr>
<th>Species</th>
<th>carp</th>
<th>carp</th>
<th>Indian medaka</th>
<th>zebrafish</th>
<th>three-spined stickle</th>
<th>rainbow trout</th>
<th>tilapia</th>
<th>killifish</th>
<th>human</th>
<th>mouse</th>
<th>rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japanese eel CYP1B1</td>
<td>66</td>
<td>66</td>
<td>66</td>
<td>62</td>
<td>62</td>
<td>69</td>
<td>67</td>
<td>65</td>
<td>57</td>
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<td>57</td>
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<tr>
<td>carp CYP1B1</td>
<td>51</td>
<td>63</td>
<td>64</td>
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<td>60</td>
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<tr>
<td>carp CYP1B2</td>
<td>63</td>
<td>84</td>
<td>61</td>
<td>64</td>
<td>60</td>
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<td>59</td>
<td>57</td>
<td>57</td>
<td>54</td>
<td>55</td>
</tr>
<tr>
<td>Indian medaka CYP1B1</td>
<td>60</td>
<td>74</td>
<td>67</td>
<td>67</td>
<td>76</td>
<td>74</td>
<td>50</td>
<td>57</td>
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<tr>
<td>zebrafish CYP1B1</td>
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<td>61</td>
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<tr>
<td>three-spined stickle CYP1B1</td>
<td>60</td>
<td>70</td>
<td>76</td>
<td>60</td>
<td>66</td>
<td>58</td>
<td>59</td>
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<td>59</td>
</tr>
<tr>
<td>rainbow trout CYP1B1</td>
<td>70</td>
<td>83</td>
<td>74</td>
<td>74</td>
<td>85</td>
<td>54</td>
<td>53</td>
<td>53</td>
<td>53</td>
<td>53</td>
<td>53</td>
</tr>
<tr>
<td>Nile tilapia CYP1B1</td>
<td>74</td>
<td>56</td>
<td>56</td>
<td>56</td>
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<td>56</td>
<td>56</td>
<td>56</td>
<td>56</td>
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<tr>
<td>killifish CYP1B2</td>
<td>73</td>
<td>59</td>
<td>59</td>
<td>59</td>
<td>59</td>
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<tr>
<td>human CYP1B1</td>
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<td>92</td>
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<td>92</td>
<td>92</td>
<td>92</td>
<td>92</td>
<td>92</td>
</tr>
</tbody>
</table>

Phylogenetic analysis

The phylogenetic tree based on the amino acid sequences of \textit{CYP1B} genes of teleost and mammalian species is shown in Figure 3. The higher similarity (69\%) of the deduced amino acid sequences between Japanese eel \textit{CYP1B1} and that of rainbow \textit{CYP1B1} resulted in the clustering of the two genes in one branch apart from other \textit{CYP1B} genes.

Fig. 3: Phylogenetic tree of \textit{CYP1B} subfamily genes constructed by pair group unweighted method (UPGM) using the amino acid sequences of teleosts and mammals.

CYP1B1 mRNA level in different tissues of BNF treated Japanese eel

QRT-RCR results revealed that there was a large increase in CYP1B1 mRNA in gills (539.56 fold), followed by liver (71.93), and kidney (3.78) while the constitutive expression was observed with the induced intestine (0.062) (Table 4, Figure 4).

Table 4: QRT-PCR results for Japanese eel CYP1B1 mRNA

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean Qty</th>
<th>Std Dev</th>
<th>Fold</th>
<th>t-value</th>
<th>Pr</th>
</tr>
</thead>
<tbody>
<tr>
<td>L.cont</td>
<td>0.1309</td>
<td>0.05</td>
<td>71.93</td>
<td>8.29</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>L.ind</td>
<td>9.4163</td>
<td>1.94</td>
<td>539.56</td>
<td>8.42</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>K.cont</td>
<td>5.0478</td>
<td>0.45</td>
<td>3.78</td>
<td>3.12</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>K.ind</td>
<td>19.0766</td>
<td>7.77</td>
<td>539.56</td>
<td>-7.45</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>G.cont</td>
<td>0.0025</td>
<td>0.00</td>
<td>539.56</td>
<td>8.42</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>G.ind</td>
<td>1.3489</td>
<td>0.28</td>
<td>3.78</td>
<td>-</td>
<td>***</td>
</tr>
<tr>
<td>I.cont</td>
<td>0.48</td>
<td>0.10</td>
<td>539.56</td>
<td>-7.45</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>I.ind</td>
<td>0.0296</td>
<td>0.01</td>
<td>0.06</td>
<td>-</td>
<td>***</td>
</tr>
</tbody>
</table>

L. cont = Liver control; L. ind = liver induced; K. cont = kidney control; K. ind = kidney induced; G. cont = gill control; G. ind = gill induced; I. cont = intestine control; I. ind = intestine induced.
Molecular characterization and organs expression of cytochrome P450 1B1 from A. japonica

DISCUSSION

The obtained nucleotide sequence of Japanese eel CYP1B1 contained an open reading frame of 1626 bp coding for 541 amino acids. Carp CYP1B1 and -1B2 ORF were found to code for 530 amino acids (El-Kady et al., 2004a, b) while the ORF of tilapia was found to code for only 531 amino acids (Hassanain et al., 2013). The ORF of the other CYP1B1 subfamily members coding for 514, 525, 526, 536, 537 and 541 for Indian medaka, three-spined stickleback fish, zebrafish, catfish, killifish and rainbow trout CYP1B1 respectively.

The phylogenetic tree based on the amino acid sequences of CYP1B of teleost and mammalian species showing the clustering of the newly identified Japanese eel CYP1B1 sequence with that of rainbow trout sequence in one branch showing a closer relationship to each other apart from other CYP1Bs.

In the present study, QRT-PCR results revealed that there was a large increase in CYP1B1 mRNA in gills (539.56 fold), followed by liver (71.93), and kidney (3.78) while the constitutive expression was observed with the induced intestine (0.062). Concerning CYP1B1 expression levels in other fish species, studies on plaice CYP1B1 expression reported more restricted tissue expression profile, only being detectable in gill tissue suggesting a role in gas and fluid regulation (Leaver & George, 2000). Carp exposed to 3-MC had CYP1B1 messenger RNA expression in liver, intestine, and gill, while CYP1B2 was only induced in the gills (El-Kady et al., 2004a, b). Similarly, BaP-exposed catfish had significant CYP1B1 mRNA induction in blood, liver, and gonad tissues while the high constitutive expression was detected in gill (Willett et al., 2006). The Constitutive expression of CYP1B1 was restricted to gills of Trematomus bernacchii, an Antarctic fish where it was also induced by BNF (Di Bello et al., 2007). Dorrington et al., (2012) stated that the induction of Brazilian guppy with 3-MC revealed the strong induced expression of CYP1B1 in the gill. Also the same result was observed with the expression of mRNA of three-spined stickleback fish (Gasterosteus aculeatus) that revealed the induction of CYP1B1 and CYP1C1 in gills at all concentrations of effluent from drug manufacturing while effects on these genes in liver and brain were weak or absent (Beijer et al., 2013). On the other hand, Hassanain et al., 2013 reported that after intracoelomic injection with BaP, the
expression of CYP1B1 in Nile tilapia revealed a large increase in CYP1B1 mRNA in liver (22.8), intestine (2.0) and muscles (1.3). In adult zebrafish, the eye, the brain and the heart are the organs with the highest basal expression of CYP1B1 (Jönsson et al., 2007b). There is also a high basal expression of CYP1B1 in zebrafish embryos, possibly related to the development of the eye, brain or heart (Jönsson et al., 2007a).

Studies on human CYP1B1 revealed its expression constitutively in extrahepatic organs, including fetal tissue samples and is highly inducible in various organs by 2,3,7,8-tetrachlorodibenzo-p-dioxin and related compounds in experimental animal models (Shimada et al., 1996). CYP1B1 is also expressed in the eye in mammals, and is essential for normal eye development (Choudhary et al., 2006).

**CONCLUSION**

This study provides the first description of the molecular characterization of Japanese eel CYP1B1 and the relationship of this newly identified sequence with that of the previously reported CYP1B subfamily members of fish and mammals. Also the higher induction of Japanese eel CYP1B1 mRNA in gills; the tissue in direct contact with the environment, followed by liver and kidney, may have important implication for the possible endogenous functions in these organs.

**REFERENCES**


**ARABIC SUMMARY**

التوصيف الجيني وقياس التعبير الجيني للسيتوكروم P450 1B1 من أعضاء ثعبان السمك الياباني (Anguilla japonica)

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تعتبر عائلة السيتوكروم P1، إحدى العائلات الجينية للقصيلة CYP، ولها حتى الآن أربع تحت عائلات موضعة في بنك الجينات / EMBL ، و CYP1D1، CYP1C، CYP1B1، CYP1A  هي: CYP1B1  كمحل فارغ يولد في الحساسية CYP1B1  وذلك من CYP1B1  وسجل تحت اسم CYP1B1  في تطور السمك الياباني بعد الحفن داخل الوعاء البروتيني بمادة Naphthoflavone، واكتسب هذا الحمض 495 قاعدة نبتوجينية، منها 162 قاعدة تمثل منطقة "السماكة المترerah" والتي تبدأ TAA وتعزز هذه الأحماض TAA  وتتشابه 44 حمض أميني وتتناسب بنك الوراثة ATG، وتشابه هذه الأحماض CYP1B1  في تطورات زمنية 770 كيلو دالتون. أظهر تسلسل الأحماض الأمينية لروتين CYP1B1  لأسماك three-spined stickle back  المستخرجة من ثعبان السمك الياباني 22٪ تشابه مع CYP1B1  من العائلة والذئب. كما أظهر نسبة تشابه بلغت 76٪ مع كل من أسماك الكوكي فيش، الميداكا اليابانية، وأسماك المرور الشائع CYP1B1 وB2  بينما لوحظ أعلى نسبة تشابه للأحماض الأمينية (77 ، 74٪) مع أسماك البلطي الشائع CYP1B1  وتراوت قوس قرف على التوازي. كما أظهر هذا المعزول من ثعبان السمك الياباني CYP1B1  نسبة تشابه مع الجينات المزعولة من الثدييات CYP1B1  كانت 57، 56٪ للإنسان، جرذان CYP1B1s، والفتران المنزلي على التوازي.

كان تتابع الجين الممزول من ثعبان السمك الياباني يتماشى مع جينات عائلة CYP1  وتم إعداده في بنك الجينات EMBL تحت رقم AR518340 . أظهرت شجرة التشابه والتطور باستخدام متابعة جينات CYP1B1  تحت عائلة CYP1B1  المعزول من الأسماك المذكورة سابقاً من الثدييات و الأسماك، وتبين أن الجين الجديد قريب إلى حد كبير (QRT-PCR) مراقبة قوس قرف CYP1B1  وبإجراء تحليلات تفاعل الفيرونا المتسلسل الكمي (QRT-PCR) لأسماك ثعبان المكسيكية،، ونلاحظ تشابه الأحماض الأمينية لثعبان السمك الياباني وjasmine والذئب،، وميزة بلغت قيمته 3،87، و 2،93، 0،52، 0،43، 0،33 على التوالي.

Molecular characterization and organs expression of cytochrome P450 1B1 from A. japonica