Activation of the aryl hydrocarbon receptor (AHR) by toxic polyhalogenated aromatic hydrocarbons like 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) leads to the transcriptional induction of genes in a CYP1A gene subfamily as well as UDP-glucuronosyl-transferase and glutathione S-transferases, and to a toxicity syndrome that includes cardiac dysfunction, carcinogenicity or congenital malformations like cleft palate in rodents [14]. Fish embryos are highly sensitive to TCDD and show circulation failure, edema, craniofacial malformation and death [12]. It has been reported that in fish embryos CYP1A can be induced by TCDD and that there is a correlation of that induction with TCDD-induced toxicity [2, 5, 10]. However, detailed localization of CYP1A mRNA and protein expressions, especially in their relationship, remains to be determined in early fish embryos. Zebrafish (Danio rerio) is growing as a model organism for environmental toxicology [7]. The abundant molecular background and thousands of artificially produced mutant lines are available, in addition to the ability to spawn hundreds of embryos and that develop rapidly (hatch in 2 days after fertilization). Besides, zebrafish is ideal system for easy application of forced expressions of specific gene or gene knock-down, by injection of mRNA or morpholino antisense mRNA into 1-8 cell stage embryo. In the present study, we determined the cDNA sequence of the open reading frame with both 5'- and 3'-ends in zebrafish (zfCYP1A), a useful model for environmental toxicology. zfCYP1A shows high percentage identity with CYP1As of mammals, domestic fowl and xenopus (51.9–60.4%), as well as the other fish species (63.8–89.2%). As revealed by in situ hybridization and immunohistochemistry, zfCYP1A was scarcely detected in control embryos but was markedly induced by TCDD especially in heart, vascular endothelial cells, intestinal epithelium, pronephros and outer integument in both prehatched and hatched embryos. These expression patterns are consistent with possible involvement of zfCYP1A in TCDD-induced toxicities.

**NOTE Toxicology**

cDNA Cloning and Expressions of Cytochrome P450 1A in Zebrafish Embryos

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**ABSTRACT** Cytochrome P450 1A (CYP1A) is well known for being induced by aromatic hydrocarbons, including 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). We determined the complete cDNA sequence of a CYP1A open reading frame with both 5’- and 3’-ends in zebrafish (zfCYP1A), a useful model for environmental toxicology. zfCYP1A shows high percentage identity with CYP1As of mammals, domestic fowl and xenopus (51.9–60.4%), as well as the other fish species (63.8–89.2%). As revealed by in situ hybridization and immunohistochemistry, zfCYP1A was scarcely detected in control embryos but was markedly induced by TCDD especially in heart, vascular endothelial cells, intestinal epithelium, pronephros and outer integument in both prehatched and hatched embryos. These expression patterns are consistent with possible involvement of zfCYP1A in TCDD-induced toxicities.

**KEY WORDS** CYP1A, TCDD, zebrafish.
Fig. 1. Nucleotide and deduced amino acid sequences of cytochrome P450 1A (zfCYP1A) of zebrafish. The numerals at the end of each line refer to the nucleotide positions. The amino acid sequence in box indicates the putative heme binding region. Underline indicates the sequence used for probes for \textit{in situ} hybridization.
CYP1A5 (TCDDAA) of chick (Gallus gallus) the discrepancy between amino acid similarity and function, zfCYP1A to CYP1A1 or CYP1A2 forms. As an example of required to further define the functional relationships of mammalian CYP1A1 or CYP1A2 clusters. In comparison with CYP1A1s of various species, the deduced amino acid sequence of zfCYP1A exhibited high percentage identity with mammalian CYP1As of various species, the deduced amino acid sequence, the deduced amino acid sequence of CYP1A5 is more like mammalian CYP1A1 than CYP1A2 [4].

With the use of the cloned zfCYP1A fragment, we have detected similar patterns of CYP1A mRNA expression in zebrafish embryos. As shown in Fig. 2A, expression of zfCYP1A mRNA was almost absent or very weak in control embryos. However, it was markedly induced following TCDD treatment (1 ppb, Fig. 2B), at a TCDD concentration that produced circulation failure and apoptosis in dorsal midbrain as well as retardation of lower jaw development in zebrafish embryos [3, 6, 16]. Extensive zfCYP1A mRNA induction by TCDD could be observed throughout stages we examined (30–72 hpf). Subsequently, we determined the site of expression by in situ hybridization together with immunohistochemistry. Similar to the result of in situ hybridization, CYP1A-immunoreactivity could rarely be detected in control embryos, but was markedly induced by TCDD treatment in both prehatched (48 hpf) and hatched embryos (60 hpf) (Fig. 2D). As shown in Fig. 2, zfCYP1A was expressed in vascular endothelium (E, F), heart (J), lower jaw primordia (I), intestinal epithelium (G, H), and outer integument (D, I, J). In addition, extensive expression of zfCYP1A was also detected in notochord and pectoral fin bud. Expression patterns of zfCYP1A mRNA were quite coincident with CYP1A protein determined by immunohistochemistry for all tissues (Fig. 2E and F; G and H). For control embryos at 60 and 72 hpf, epithelium of intestine showed zfCYP1A expressions. Robust expression of zfCYP1A in epithelium rather than mesenchymal cells in lower jaw primordia (Fig. 2I) [16], is reminiscent of the expression pattern of AhR2 in lower jaw primordia, and therefore raises the possible involvement of zfCYP1A in retarded jaw growth by TCDD. Similarly, extensive expression of zfCYP1A in vascular endothelium and heart could be related to TCDD-induced circulation failure including edema. It has been reported that an AHR agonist, PCB77 induces albumin permeability, concomitant with CYP1A induction in cultured porcine endothelial cells [17]. Apoptosis in intestinal epithelium stimulated by TCDD has been reported in xenopus embryos [15]. However, zfCYP1A expressions in intestinal epithelium could also be detected in control embryos from 60 hpf, as mentioned above. Some results similar to ours on the localization of CYP1A by in situ hybridization and immunohistochemistry have been obtained by R.E. Peterson and his colleagues (manuscript in preparation).

We have found only one zfCYP1A clone. Some fish have multiple CYP1A genes, such as rainbow trout (rtCYP1A1 and rtCYP1A3). However, these two rtCYP1As are very similar in their nucleotide sequences and show high % identity in their coding region (97.4%) [13]. Furthermore, we used a conserved region for the hybridization probe. Therefore, we assume that we would have detected both of two zfCYP1As together, if indeed there is another CYP1A gene expressed in zebrafish. Just recently, Mattingly and Toscano cloned partial fragment of zfCYP1A and showed that zfCYP1A mRNA was induced by TCDD using northern blot procedures with a whole body homogenate [9]. However, they reported that CYP1A protein was not induced before hatching, in contrast to our results.

In summary, we have detected similar patterns of CYP1A mRNA and protein expression in embryonic zebrafish exposed to TCDD. These zfCYP1A expression patterns support a possible involvement of CYP1A in the various toxicities by TCDD. Application of forward genetic method available for zebrafish embryos such as morpholino antisense gene knock-down methods, which requires information of cDNA 5’-sequence, could lead us to clarify the role of CYP1A in TCDD toxicity.

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REFERENCES


Fig. 2. Expression patterns of mRNA and immunoreactivity of zfCYP1A in zebrafish embryos. Zebrafish embryos were treated with (B, D-J) or without (Control: A, C) 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, 1 ppb), started from 24 hr post fertilization (hpf) and fixed at 48 hpf (before hatching) for whole-mount in situ hybridization (A, B, E, G) or immunohistochemistry (C, D, F, H, I, J). Arrows in F (dorsal aorta), I (lower jaw promordia) and J (cardiac ventricle) represent CYP1A immunoreactivity. Arrow heads in C and F indicate pigment in outer integument and erythrocytes in dorsal aorta, respectively. Bars = 500 µm (A, B), 100 µm (C, D, I, J) and 10 µm (E-H).