The aryl hydrocarbon receptor (AHR) forms part of a transcription complex upon activation by xenobiotic ligands such as TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin), a toxic environmental contaminant. Frogs exhibit low sensitivity to TCDD compared to other vertebrates, which is attributed to the weak affinity of frog AHRs to TCDD. This lowered affinity has been traced to specific amino acids within the ligand-binding domain of the frog AHR (Odio et al, 2013). In this study, we seek to determine whether low TCDD affinity is unique to frog AHRs or a property shared by other amphibian groups. We cloned an AHR cDNA sequence from the Mexican axolotl salamander (Ambystoma mexicanum) via RT-PCR with degenerate primers and RACE-PCR. Phylogenetic analysis indicates that the salamander AHR is of the AHR1 lineage, as are the AHRs of frogs. The A. mexicanum AHR possesses the same amino acids in the ligand-binding domain that confer lowered TCDD affinity to frog AHRs. Based on this sequence characterization, we predict that salamanders, like frogs, are relatively insensitive to TCDD. A reporter gene assay determining the ability of the A. mexicanum AHR to regulate the expression of target genes has been optimized, and preliminary results suggest that the salamander AHR does not induce TCDD-dependent gene expression as readily as a mammalian AHR, which binds TCDD with high affinity.

Methods

- **Cloning of a partial AHR cDNA**: A 600 bp portion of the A. mexicanum AHR was amplified via RT-PCR using degenerate primers (sense 5’-CGGGATCCGAYTAYCTIGGITTYCARCA-3’, antisense 5’-GCTCTAGACATICCRCTYTCICCIGTYTT-3’) designed to target conserved regions of the vertebrate AHR amino acid sequence. This amplicon (Figure 1a) was cloned into the pGEM-T Easy vector and sequenced.

- **Obtaining the full length AHR sequence**: Using the partial cDNA sequence, AHR-specific primers were designed to target the 5’ and 3’ ends of the cDNA for use in RACE (Rapid Amplification of cDNA Ends) PCR. The 5’ and 3’ amplicons (Figure 1b) were cloned into the pGEM-T Easy vector and sequenced. The clones were aligned together with the partial cDNA, providing the entire AHR sequence (Figure 2).

- **Phylogenetic analysis**: The predicted amino acid sequence of A. mexicanum AHR was aligned with the protein sequence of other vertebrate AHRs using the ClustalW2 online tool and rooted with the AHR homolog from the chicken. Phylogenetic trees were created using the Neighbor-Joining method and rooted with the AHR homolog from the chicken. The phylogenetic tree was inferred by the Neighbor-Joining method, and rooted with the AHR homolog from the chicken. The tree topology is consistent with the tree topology of other AHRs from different species, indicating that the A. mexicanum AHR is closely related to the AHRs from other vertebrates.

- **Constructing an AHR expression vector**: The open reading frame of the A. mexicanum AHR was synthesized by Epoch Life Sciences and subcloned into the pCMV-TNT vector.

- **Transactivation assay**: The responsiveness of the A. mexicanum AHR to TCDD was characterized with a reporter gene assay measuring its ability to induce the expression of target genes. The AHR expression construct was co-transfected with Lipofectamine 2000 into COS-7 cells along with an ARNT expression construct, a firefly luciferase reporter plasmid, and a Renilla luciferase translation control plasmid. For comparison with other AHRs, cells were also transfected with a frog AHR (Xenopus laevis AHR1β) or a chimeric AHR (X. laevis AHR1β with ligand binding domain of mouse AHR). After transfection, cells were exposed to graded concentrations of TCDD. Transactivation by the AHR was determined by measuring the luciferase activity using the Dual Luciferase Kit (Promega) and a TD-20/20 luminometer (Figure 5).

- **Prediction**: Salamanders are relatively insensitive to TCDD toxicity.

**Conclusion**

The salamander AHR exhibits reduced responsiveness to TCDD than a mouse-like AHR in its ability to induce target genes. Our characterizations predict that salamanders, like frogs, are relatively insensitive to TCDD toxicity. Future studies include determining the binding affinity of the A. mexicanum AHR to TCDD, and measuring its responsiveness to FICZ, an endogenous AHR ligand.

**References**


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