Identification of a Novel Dioxin-Inducible Cytochrome P450

STEVEN P. RIVERA, SIRKKU T. SAARIKOSKI, and OLIVER HANKINSON
Department of Pathology and Laboratory Medicine, Jonsson Comprehensive Cancer Center, and Molecular Biology Institute, UCLA, Los Angeles, California

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ABSTRACT

Representational difference analysis was used to isolate cDNAs corresponding to 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin)-inducible genes from mouse Hepa-1 cells. One cDNA encoded a novel cytochrome P450. The human homolog was also isolated and later proved to be human CYP2S1. The induction of mouse CYP2S1 mRNA by dioxin represents a primary response and required the aryl hydrocarbon receptor and aryl hydrocarbon receptor nuclear translocator proteins. The induction of CYP2S1 also occurred in mouse liver and lung, with the highest expression found in lung. CYP2S1 was also inducible in a human lung epithelial cell line. The dioxin-inducibility of CYP2S1 is exceptional, because all previously well-characterized cases of the induction of cytochromes P450 by dioxin involve members of the CYP1 family.

Materials and Methods

Chemicals and Reagents. Dioxin was obtained from the National Cancer Institute Chemical Carcinogen Repository (Bethesda, MD). Dimethyl sulfoxide (DMSO) and cycloheximide were purchased from Sigma-Aldrich (St. Louis, MO).

Cells and Cell Culture. Mutant strains of the mouse hepatoma cell line Hepa-1c1c7 (Hepa-1) that are deficient in ARNT (c4) and AHR (c12) were constructed in this laboratory previously (Hankinson, 1995). In contrast to PAHs and aromatic amines, dioxin is refractory to biotransformation, and the parent compound itself acts as a carcinogen. The induction of CYP1A1 has been well-studied. After binding the ligand, the AHR translocates to the nucleus, where it dimerizes with the ARNT protein. The AHR/ARNT dimer then binds to xenobiotic-responsive elements in the 5′ flank-

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S.P.R. and S.T.S. contributed equally to this work.

ABBREVIATIONS: P450, cytochrome P450; PAH, polycyclic aromatic hydrocarbon; dioxin, 2,3,7,8-tetrachlorodibenzo-p-dioxin; RDA, representational difference analysis; DMSO, dimethyl sulfoxide; PCR, polymerase chain reaction; NCBI, National Center for Biotechnology Information; EST, expressed sequencer tag; RACE, rapid amplification of cDNA ends; Hepa-1, hepatoma cell line Hepa1c1c7; ChoB, Chinese hamster ovary B; AHR, aryl hydrocarbon receptor; ARNT, aryl hydrocarbon nuclear translocator; XRE, xenobiotic-responsive element.
Results

Isolation of a Novel Dioxin-Inducible Cytochrome P450. An RDA experiment performed on the Hepa-1 cell line comparing cDNAs from untreated cells and cells treated for 24 h with dioxin led to the isolation of several dioxin-inducible clones. This report focuses on one of these clones. Data-bank searches with the sequence of this RDA clone revealed a series of continguously overlapping ESTs. The assembled sequence for this set of ESTs indicated that this clone represented a previously uncloned cytochrome P450. Further data-bank analyses also indicated the existence of a human homolog for this gene. Because the sequence data available in the EST database covered only the C-terminal end of these putative new P450s, 5'-RACE was used to obtain the full-length coding sequence of both the mouse and human cDNAs. The human cDNA we identified proved later to be identical with human CYP2S1 (Rylander et al., 2001). Therefore CYP2S1 cDNAs were labeled by random primed [32P]dATP incorporation (Prime-A-Gene Labeling Kit; Promega). Phosphorimaging analysis was performed by the use of a 455SI PhosphorImager (Molecular Dynamics, Sunnyvale, CA) with correction for interlane load variability by comparison with ChoB, a constitutively expressed gene that is not responsive to dioxin or cycloheximide. ChoB cDNA was kindly provided by Dr. Harvey Herschman (UCLA, Los Angeles, CA).

In Vitro Transcription/Translation. In vitro transcription/translation was performed using PCR products of the mouse and human CYP2S1 full-length coding regions. To allow transcription from the PCR product, 5' primers containing the T7 promoter sequence were designed. Expression was achieved using the Tnt T7-coupled reticulocyte lysate system according to the manufacturer's instructions (Promega) in the presence of [35S]methionine (ICN, Costa Mesa, CA). Protein products were analyzed on a 10% SDS-polyacrylamide gel, and the size of the protein was determined by comparison with a standard size marker (Bio-Rad, Hercules, CA).
called our mouse and human homologs CYP2S1. Alignment of human and mouse CYP2S1 amino acid sequences showed that the proteins are 78% identical (Fig. 1). The cDNAs are 81% identical in nucleotide sequence. Mouse CYP2S1 exhibits 49% identity with mouse cytochromes CYP2G1 and CYP2B10, whereas the closest relatives of human CYP2S1 are members of the CYP2A and CYP2B subfamilies (CYP2A6, CYP2A13, and CYP2B6), which show 49% homology with CYP2S1.

Full-length cDNAs for both mouse and human CYP2S1 were generated by PCR downstream from a T7 promoter sequence and expressed by in vitro transcription/translation. The proteins generated were both 56 kDa compared with standard-size markers (data not shown). This is the appropriate size predicted from the amino acid sequences of the two proteins.

Characterization of mCYP2S1 Induction by Dioxin in Mouse Cells. The mouse CYP2S1 mRNA detected in Hepa-1 cells is approximately 2.6 kb. Induction of this mouse CYP2S1 mRNA by 10 nM dioxin in Hepa-1 cells was evident in 3 h, was maximal at 6 h, and declined thereafter (Fig. 2A). The average maximal induction was 10-fold. Induction by dioxin was not blocked by cycloheximide (a protein-synthesis inhibitor) at a concentration 10-fold greater than that known

![Image]

**Fig. 2.** Northern blotting analyses of mCYP2S1 expression in mouse Hepa-1 cells. A, time course of induction of mCYP2S1. Hepa-1 cells were treated with 10 nM dioxin for the indicated times or with vehicle (DMSO) only (Ctl). ChoB, a constitutively expressed gene that does not respond to dioxin or cycloheximide, was used to correct for interlane load variability. B, effect of cycloheximide on mCYP2S1 induction. Hepa-1 cells were treated with dioxin (10 nM), cycloheximide (CHX, 100 μM), or both for 6 h. C, dose-response curve for the induction of mCYP2S1 and mCYP1A1 by dioxin. Hepa-1 cells were treated with various concentrations of dioxin for 6 h, and mRNA was subjected to Northern blot analysis using CYP2S1, CYP1A1, and ChoB probes. CYP2S1 and CYP1A1 mRNA levels were quantitated by PhosphorImaging analysis and corrected for the amounts of ChoB mRNA. The figure represents the result from two independent experiments, except for the data for 0.0001 and 0.0003 nM dioxin, each of which was obtained from a single experiment. D, induction of mCYP2S1 in response to 10 nM dioxin in wild-type Hepa-1 cells and mutant cell lines deficient in expression of AHR (c12) or ARNT (c4) or in the mutant expressing AHR with impaired XRE binding activity (c35).
to inhibit protein synthesis in Hepa-1 cells by 95 to 97% (Israel and Whitlock, 1983). This indicates that dioxin induction of mCYP2S1 is a primary response. Cycloheximide treatment resulted in superinduction of mCYP2S1, a phenomenon that also occurs for the dioxin-inducible CYP1A1 gene (Israel et al., 1985) (Fig. 2B). Dose-response analysis for the induction of CYP2S1 and CYP1A1 mRNAs in Hepa-1 cells demonstrated that the EC$_{50}$ for the induction of CYP2S1 (0.17 nM) by dioxin is 10-fold greater than that for CYP1A1 (0.017 nM) (Fig. 2C). No induction of CYP2S1 in response to dioxin occurred in AHR- and ARNT-deficient derivatives of Hepa-1 cells, indicating that the induction of mouse CYP2S1 requires the presence of both AHR and ARNT (Fig. 2D).

**CYP2S1 Induction in Mice.** CYP2S1 mRNA induction occurred in the liver of C57BL/6 mice injected intraperitoneally with 30 µg/kg dioxin (Fig. 3A). The induction of CYP2S1 was most pronounced in the lung compared with induction in the liver, heart, kidney, and spleen (Fig. 3B). The Northern blot in Fig. 3B was exposed for a considerably shorter time than that for Fig. 3A so as not to overexpose the lanes containing the lung samples. Consequently, little or no signal was detectable for the liver samples in Fig. 3B.

**Expression of CYP2S1 in Human Cells.** The human lung cell line A549 exhibited much higher levels of the approximately 2.6-kb CYP2S1 mRNA than did the hepatoma cell lines HepG2 and Hep3B and the breast cancer cell line MCF-7. Dioxin treatment resulted in approximately a 2-fold induction of CYP2S1 mRNA in the A549 cell line (Fig. 4A). The expression of CYP1A1 exhibited an inverse pattern of expression compared with CYP2S1 in these cell lines (Fig. 4A).

**Discussion**

We describe a novel cytochrome P450 whose mRNA is inducible by dioxin. This cytochrome P450, CYP2S1, is exceptional in that other well-characterized dioxin-inducible P450s all belong to the CYP1 family, although hamster CYP2A8 has previously been shown to be inducible by PAHs in a XRE-dependent process and may therefore represent another dioxin-inducible CYP2 family member (Kurose et al., 1999).

The EC$_{50}$ of dioxin for the induction of mCYP2S1 mRNA in Hepa-1 cells was 10-fold greater than that for CYP1A1 mRNA. The reason for this difference is not currently known, although it is clear from the results with the Hepa-1 mutant cell lines that, like CYP1A1, AHR and ARNT are required for the induction of CYP2S1. The EC$_{50}$ for the induction of CYP1B1 by dioxin in rat liver has been shown to be 24-fold greater than that for CYP1A1 (Santostefano et al., 1997).

The level of induced m2S1 mRNA was much greater in the lung than in the other mouse tissues examined, including the liver. CYP2S1 mRNA was also found to be 2-fold inducible in the human lung cell line A549, whereas both uninduced and induced levels were low in the human hepatoma and breast cancer cell lines examined. Thus, although CYP2S1 mRNA was identified via its induction in mouse hepatoma cells, it seems to be expressed only weakly in normal mouse liver and human hepatoma cells. Among the limited set of human cell lines examined, a reciprocal relationship between CYP2S1 and CYP1A1 inducibility was observed. It is of interest to examine additional human cell lines to determine whether there is one in which CYP2S1 is more dramatically inducible by dioxin. Rylander and coworkers (2001) recently reported that CYP2S1 is constitutively expressed in several human tissues, including the lung. Because CYP2S1 is inducible by dioxin, we speculate that, like the other known dioxin-inducible P450s, it is capable of metabolizing important classes of chemical carcinogens. In addition, because of the high levels of CYP2S1 in dioxin-treated mouse lung and in the human lung cell line A549, we also speculate that it may play an important role in carcinogen metabolism in the lung, a major target tissue for a number of environmental carcinogens.
including tobacco smoke. Consequently, the identification of substrates for CYP2S1 is of considerable interest.

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Address correspondence to: Oliver Hankinson, Ph.D., Department of Pathology and Laboratory Medicine, UCLA Center for the Health Sciences, 10833 Le Conte Ave., P.O. Box 951732, Los Angeles, CA 90095-1732. E-mail: ohank@mednet.ucla.edu