THE MOLECULAR MECHANISM OF ACTION OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN AND RELATED HALOGENATED AROMATIC HYDROCARBONS

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OVERVIEW

Halogenated aromatic hydrocarbons (HAHs) represent a diverse group of highly toxic environmental contaminants and include such compounds as polychlorinated and polybrominated dibenzo-p-dioxin, dibenzofurans and biphenyls. These compounds have generated considerable concern because of their resistance to biological and chemical degradation, ubiquity, fat solubility, long biological half-life and extremely high toxicity. The prototypical and most potent member of this class of compounds is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, dioxin). TCDD and related HAHs produce a number of common species- and tissue-specific toxic and biological effects, including: tumor promotion, a wasting syndrome, mortality, immunotoxicity, hepatotoxicity, porphyria, dermal toxicity and induction of numerous enzymes, including microsomal cytochrome P450IA1 and its associated monooxygenase activity, aryl hydrocarbon hydroxylase (AHH) (1,2). The cytochrome P450IA1 isozyme contributes to the metabolic activation and detoxification of polycyclic aromatic hydrocarbons, such as benzo(a)pyrene, some of which are widespread environmental carcinogens (3).

The induction of hepatic AHH activity is perhaps the best studied of the biochemical effects resulting from exposure to these compounds. Early experiments examining the induction of AHH activity by a series of TCDD congeners revealed a relationship between the structure of a compound and its ability to induce AHH activity. These results, in combination with those obtained using various inbred strains of mice, suggested the presence of a specific receptor which recognized these compounds (4). A subsequent study (5) identified a cytosolic protein which bound TCDD saturably and with high affinity and which exhibited the properties of a receptor. Structure activity analysis studies have also revealed that the ability of compounds to bind to this receptor correlated well with their ability to induce AHH activity (2,5,6). Additional studies also revealed a good correlation between the ability of these compounds to bind to the receptor and their ability to induce toxicity (specifically thymic involution, wasting syndrome and epidermal keritinization) in animals was observed (1,2), supporting a common mechanism of action of these compounds. This "TCDD (or dioxin) receptor" has been identified in a wide variety of species and tissues (8) and has also been designated the Ah (for aromatic hydrocarbon responsiveness) receptor (AhR).

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The current model for the mechanism of induction of cytochrome P450IA1 (and AHH activity) by these compounds is similar to that described for steroid hormone receptors and steroid-responsive genes (8,9). Following ligand (TCDD) binding, the AhR, like steroid hormone receptors, undergoes a poorly defined process of transformation, during which hsp90 (a heat shock protein of 90 Kd) dissociates from the TCDD:AhR complex and the AhR acquires the ability to bind to DNA with high affinity (10,11). The accumulation of transformed TCDD:AhR complexes within the nucleus correlates with induction of cytochrome P450IA1 mRNA (12). The binding of transformed AhR complexes to specific DNA sequences adjacent to the cytochrome P450IA1 gene result in an increase in its rate of transcription with the subsequent accumulation of cytochrome P450IA1-specific mRNA (9,13). The translation of this mRNA into active cytochrome P450IA1 enzyme can be measured by an changes in the amount of cytochrome P450IA1-specific AHH activity. The requirement for AhR in this induction mechanism has been confirmed in studies utilizing variant mouse hepatoma cells which are defective in the induction response (9,13). We have utilized this TCDD-inducible response as a model system for studying the mechanism of action of TCDD at the molecular level.

Whitlock and coworkers (9,13) isolated DNA from the 5'-flanking region of the mouse cytochrome P450IA1 gene and inserted it directly Upstream of the bacterial chloramphenicol acetyl transferase (CAT) gene. Transfection of the recombinant plasmid into wild-type and receptor-defective variant cells revealed that CAT expression was TCDD inducible in transfected wild-type cells, while TCDD induction of CAT activity was absent or diminished when this vector was transfected into the two classes of receptor defective cells. Thus, the DNA insert contained a domain(s) with the properties expected for a TCDD-responsive DNA element. Deletion analysis (13) revealed that the 5'-flanking region of the P450IA1 gene contained a relatively strong promoter located near the start site of transcription, an inhibitory element and at least two spatially distinct and functionally independent TCDD-responsive elements, which have the properties of transcriptional enhancers and which require functional TCDD: AhR complexes for their action. These results suggested that the TCDD- responsive elements (also called dioxin-responsive elements or DREs) contained the binding site(s) for the TCDD:AhR complex.

Utilizing a sensitive gel retardation assay we have identified at least 5 DREs contained within the 5'-flanking region of the cytochrome P450IA1 gene which can specifically interact with transformed TCDD:AhR complexes (14-16). The presence of the AhR in this complex was recently demonstrated using a radioiodinated TCDD agonist (15). These DRE fragments will function independently of each other and will confer TCDD responsiveness upon a heterologous promoter and gene (14,15). Alignment of the DRE sequences from 5'-flanking region of the mouse cytochrome P450IA1 gene has revealed the following common consensus sequence present in all five DREs:

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C T C N G N C T N G C G T G N G A N N N C
T C G   A   G                 C T       G
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This consensus sequence is characterized by an invariant core sequence (TNGCGTG) as well as several variable bases flanking the core. These flanking sequences appear to be required for DRE function since their deletion significantly decreases AhR binding and/or transcriptional enhancer function (15). Similar DRE consensus sequences have been identified in the 5'-flanking region of the TCDD inducible human (18) and rat (17) cytochrome P450IA1 gene and rat glutathione S-transferase (19) gene and they appear to be functionally comparable to those we have previously identified. Additionally, transformed TCDD:AhR complexes from a wide variety of species will also bind to a synthetic oligonucleotide containing a single copy of the DRE consensus sequence (20), indicating that both the DRE consensus sequence and DNA binding domain of the AhR are highly conserved among species, Thus, the AhR, like steroid hormone receptors, appears to be a ligand-dependent DNA-binding protein, which binds to specific cis-acting DNA enhancer sequences *adjacent to a responsive gene stimulating its transcription.*

The functional role of the DRE in TCDD responsiveness suggests an obvious hypothesis which might explain the diversity of responses resulting from exposure to TCDD and related compounds. We envision that a DRE or DRE-like sequence(s) is present in the flanking region of numerous other genes and that the binding of transformed TCDD:AhR complexes to these sequences results in expression of these genes, in addition, differential expression of these genes in a species- and tissue-specific manner may also account for reported differences in TCDD responsiveness. The identification and role of other TCDD-responsive gene in these in the toxic and biological effects observed following exposure to these compounds is an exciting area of future research.

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