EROD ACTIVITY

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Measurement of ethoxyresorufin-*O*-deethylase (EROD) activity in fish is a well-established *in vivo* biomarker of exposure to certain planar halogenated and polycyclic aromatic hydrocarbons (PHHs and PAHs) and other structurally similar compounds (Bucheli and Fent 1995; Stegeman and Hahn 1994). EROD is a highly sensitive indicator of contaminant uptake in fish, providing evidence of receptor-mediated induction of cytochrome P450-dependant monooxygenases (the CYP1A subfamily specifically) by xenobiotic chemicals. Numerous laboratory experiments, simulated field studies, and natural field studies have examined EROD induction in more than 150 species of fish. In addition to PHHs and PAHs, an extensive list of individual contaminants and complex environmental mixtures has been examined for teleost EROD potential. Although EROD activity is best viewed as an indicator of exposure, the relationship between EROD and biological effects at higher levels of organization is the subject of intense investigation. It is becoming clear that the mechanism of CYP1A induction is closely related to, if not directly involved in, detrimental effects such as apoptosis and embryonic mortality seen in fish exposed to EROD-inducing contaminants (Cantrell et al. 1996). Apart from xenobiotic induction, EROD activity can be influenced by a large number of abiotic and biotic factors such as water temperature, age and reproductive phase (Andersson and Förlin 1992). An understanding of these factors is critical to the design and interpretation of field studies utilizing this biomarker.

Background

Over two decades have passed since the induction of the cytochrome P450 1A subfamily of monooxygenases (CYP1A) was proposed as a biomarker of exposure to PHHs and PAHs (Payne 1976; Payne and Penrose 1975). The foundations for this suggestion originated from the extensive work on this enzyme system performed in mammals dating back to the mid-1960s (Conney 1967; Mason et al. 1965). The

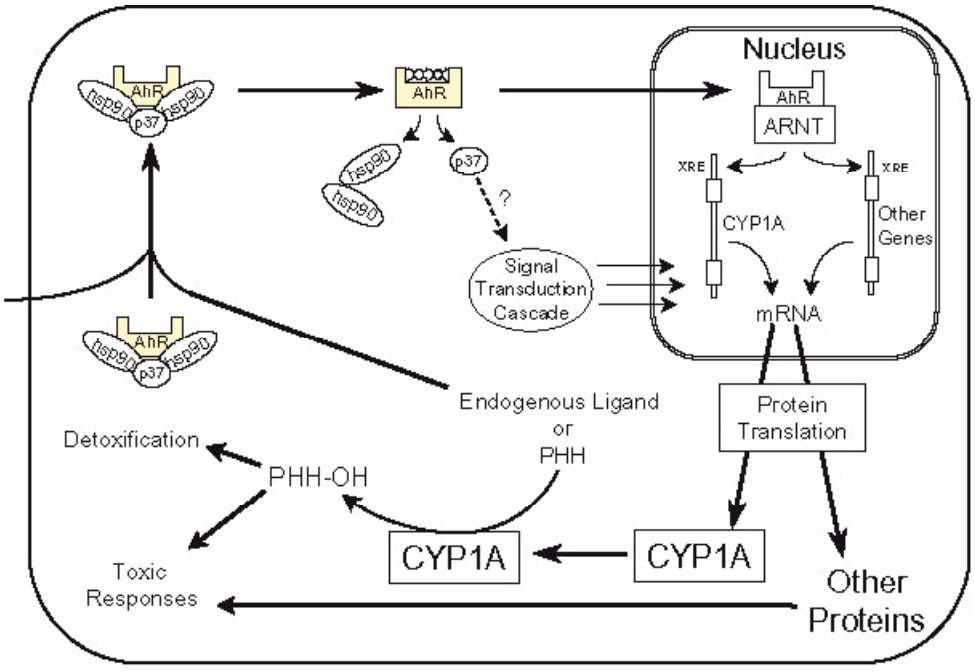

Figure 2. Proposed mechanism of AhR-mediated toxicity. Signal transduction by dioxin-like ligands is mediated by the AhR, which forms a transcription factor complex with an aryl hydrocarbon nuclear translocator protein (ARNT). This heterodimer recognizes specific DNA sequences, dioxin responsive elements (DRE), and leads to the induction of several genes (the Ah gene battery). The elevated levels of the protein products are thought to be involved in the toxic action of AhR ligands.

cytochromes P450 are a diverse multigene family of heme-containing proteins that oxidize, hydrolyze, or reduce compounds through the insertion of an atom of atmospheric oxygen to the substrate during the reaction cycle (Nebert et al. 1993; Nelson et al. 1996). In fish, these enzymes are concentrated mainly in the liver, but have been detected in the kidney, gastrointestinal tract and gill tissue (Varanasi 1989). Embedded in the smooth endoplasmic reticulum, they metabolize both endogenous and exogenous compounds (phase I reactions), generally increasing the water solubility of substrates, thereby enhancing their elimination (Andersson and Förlin 1992). In this way, cytochromes P450 such as CYP1A tend to detoxify xenobiotic chemicals; however, the phase I metabolites of some PAH and other contaminants may be more toxic than the parent compound (Guengerich and Liebler 1985).

The most useful aspect of CYP1A for biomonitoring purposes is the enzyme's tendency to increase in concentration upon chemical exposure. Induction of CYP1A is mediated through the binding of xenobiotics to a cytosolic aryl hydrocarbon receptor (AhR) (Fig. 2). AhR ligands generally have isoteric configurations and are similar in structure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD), a model CYP1A inducer (Fig. 3). Receptor binding is followed by a series of molecular events leading to the expression of several genes (including CYP1A) known as the "Ah-gene battery" (Nebert et al. 1993). The toxic effects of PHHs and structurally similar compounds are thought to be mediated through the AhR, with induced proteins causing alterations in cellular homeostasis (DeVito and Birnbaum 1994). In mammals, these effects include wasting syndrome, tumor promotion and thymic atrophy (Poland and Knutson 1982). In fish, early life stages appear to be particularly sensitive to AhR ligands (Mehrle et al. 1988; Walker and Peterson 1991), and recent evidence indicates the involvement of CYP1A enzymes specifically in this toxic response (Cantrell et al. 1996).

The use of CYP1A induction as an assessment technique has increased in recent years. This is due mainly to the optimization of protocols for the rapid and relatively inexpensive measurement of its catalytic activity as EROD (Kennedy and Jones 1994; Burke and Mayer 1974; Pohl and Fouts 1980). EROD induction as a biomarker in teleost species has several advantages. By indicating the induction of CYP1A, EROD activity provides a fingerprint of the presence of AhR-active compounds in fish. Historically, assessing the degree of uptake of these compounds was complicated by both their vast number and their varying degrees of bioavailability. Although analytical measurements can provide the identities and concentrations of organic contaminants in fish tissues (Huestis et al. 1996; Firestone 1991), they do not render a direct indication of biological potency. Induction of EROD is an extremely sensitive indicator of environmental alterations and is usually one of the first detectable, quantifiable responses to exposure (Stegeman 1992). In addition, EROD represents the cumulative impact of all inducing chemicals, whether or not they are detected analytically.

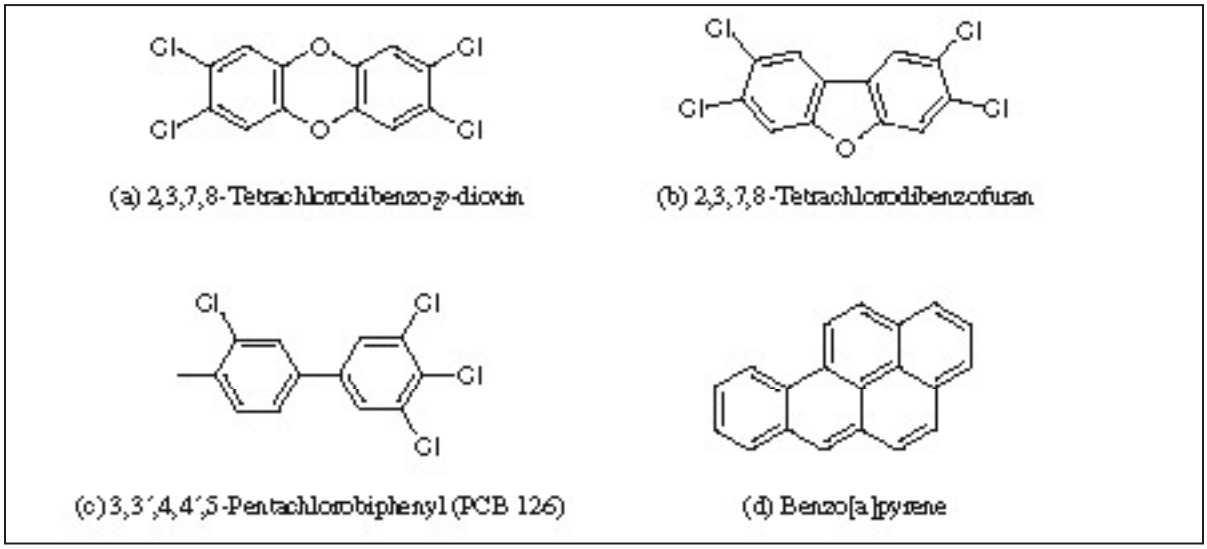

Figure 3. Representative AhR ligands. The molecules demonstrate the general structure of compounds in the following classes: (a) polychlorinated dibenzo-p-dioxins (PCDDs), (b) polychlorinated dibenzofurans (PCDFs), (c) polychlorinated biphenyls (PCBs) and (d) polycyclic aromatic hydrocarbons (PAHs).

Performing the EROD Assay

EROD activity describes the rate of the CYP1A-mediated deethylation of the substrate 7-ethoxyresorufin (7-ER) to form the product resorufin. The catalytic activity towards this substrate is an indication of the amount of enzyme present and is measured as the concentration of resorufin produced per mg protein per minute (mol/mg/min) (Kennedy and Jones 1994). Because metabolism is generally highest in hepatic tissue, the assay is typically conducted using fish liver. Determination of EROD activity involves two stages. The first stage is fish capture (typically 7-10 individuals per site) followed by the excision and cold preservation of the liver tissue. The excised tissue is homogenized and centrifuged to isolate fragments of the endoplasmic reticulum. This microsomal fraction contains the CYP1A enzymes of interest (Pohl and Fouts 1980). Consistency in terms of liver section removed, cryopreservation technique and microsomal preparation are important means of reducing individual sample variability (Pluta 1995; Heinonen et al. 1996). The second stage, the actual enzymatic assay, involves providing the microsomal fraction with 7-ER and NADPH and fluorometrically measuring resorufin production. Samples are then standardized based on protein content of the liver homogenate (Lorenzen and Kennedy 1993; Lowry et al. 1951; Bradford 1976).

Factors That Can Affect EROD Induction in Fish

As with most biological phenomena, EROD in the tissues of an organism is influenced by a variety of internal, external, and temporal factors (Bucheli and Fent 1995). Biological factors that can influence EROD activity include species (Addison et al. 1991; Segner et al. 1995), fish size and age (Peters and Livingstone 1995; Pluta 1993), and reproductive status (Campbell et al. 1976; Schreck and Hopwood 1974). The physical treatment of fish in both laboratory and field studies can also greatly affect EROD measurements. Careful consideration should be given to contaminant exposure route (James and Bend 1980; Haasch et al. 1993), fish diet (Jimenez and Burtis 1988), and the use of anesthesia during capture (Kleinow et al. 1986). Environmental variables such as temperature and pH can drastically affect induction of EROD and should be routinely measured throughout a study (Andersson and Koivusaari 1985; Sleiderink et al. 1995; Willis et al. 1991). Contaminant exposure period and study duration are important in both laboratory and caged-fish studies examining EROD (van der Weiden et al. 1994b; Sleiderink and Boon 1996).

A variety of chemicals and chemical mixtures are known to inhibit the induction of EROD in fish. These include organic, organometallic, and metallic compounds such as specific polychlorinated biphenyl (PCB) congeners (Gooch et al. 1989; Newsted et al. 1995), and organotins (Bucheli and Fent 1995). In addition to antagonistic chemicals, there a variety of AhR agonists that are of biogenic nature, including plant metabolites and biotoxins (Takahashi et al. 1995). In short, the presence or absence of EROD activity in fish from a site may not always represent contamination by or lack of traditional AhR agonists, and study designs incorporating a battery of tests for biological responses to contaminants will likely yield more concrete information.

Value and Utility of EROD in the BEST Program

As a monitoring tool, EROD activity provides a relatively rapid indication of toxic planar compound uptake in fish. For this reason, EROD is often termed an “early warning system” (Payne et al. 1987). Thus far, hundreds of field studies have employed the EROD assay to determine spatial and temporal trends of contamination in aquatic systems (Kennedy and Jones 1994; Balk et al. 1993; Teal et al. 1992; Adams et al. 1996; Achazi and Leydecker 1992). These studies have detected EROD induction in many species of fish from a broad range of habitat types. This extensive validation of EROD as a biomarker has led to its use in several contaminant monitoring programs worldwide [e.g., North Sea Task Force, (Stagg 1991; Pluta 1995), Environment/Cellulose (Förlin et al. 1995), Mediterranean Pollution Network (Burgeot et al. 1996), French National Observation Network (Godefroy et al. 1996)].

Ideally, a biomarker will exhibit a relationship to toxicity in the organism(s) being examined. Induction of CYP1A (estimated from EROD activity), while not a toxic response per se, does indicate the potential for AhR ligands to induce biochemical change. The generation of reactive PAH intermediates by CYP1A has long been known as a source of DNA adducts that can lead to carcinogenesis [Fig. 4, (Varanasi et al. 1989)]. In addition, a variety of parameters in fish have been associated with the induction of EROD activity including reproductive effects (e.g. reduced serum steroid levels), increased liver somatic index and mortality (Watson and Di Giulio 1997; van der Weiden et al. 1994a; Levine et al. 1995; Gagnon et al. 1994). At present the many symptoms and effects that AhR ligands exert on a wide variety of biochemical systems have made it difficult to identify the critical events that lead to

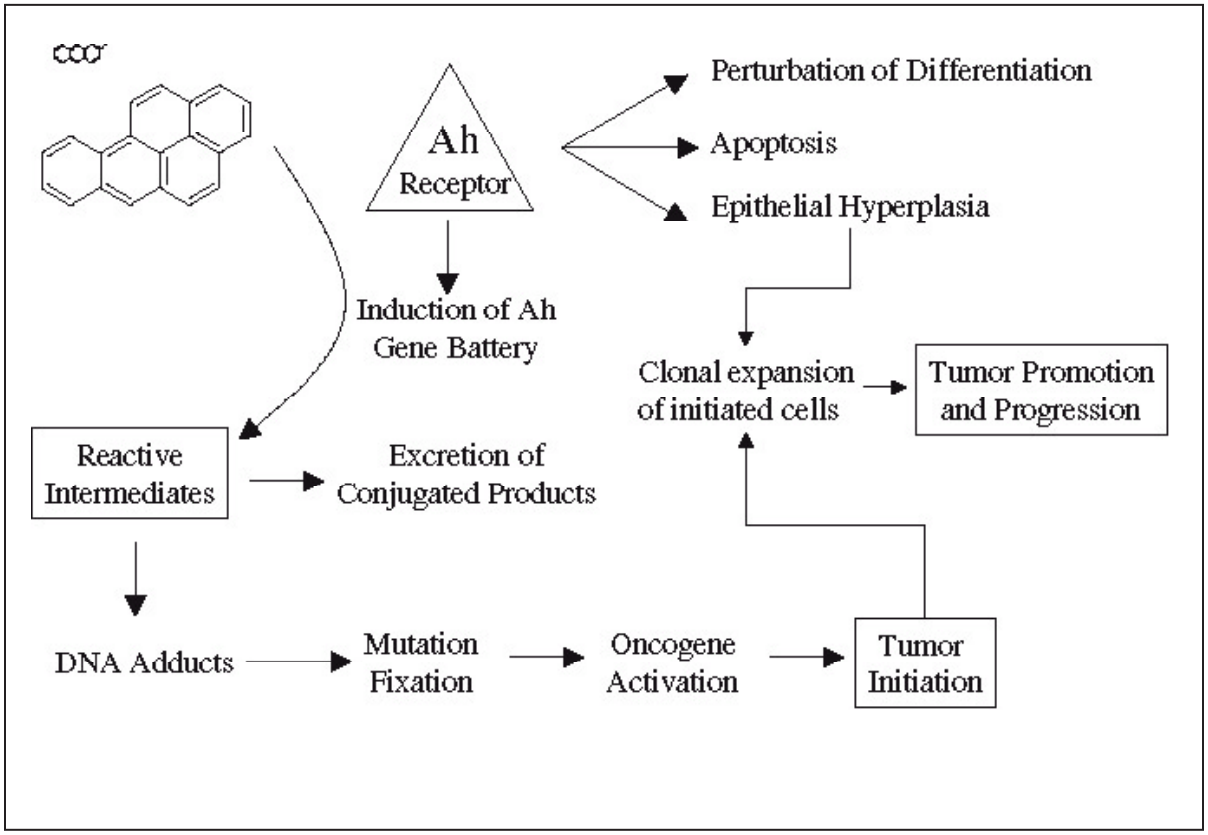

Figure 4. Ah receptor-mediated formation of DNA adducts and tumor initation upon exposure to benzo[*a*]pyrene. Compounds such as 2,3,7,8-TCDD are also capable of perturbing differentation, affecting apoptotic balance and causing tissue-specific proliferation, another hypothesized route of AhR-mediated carcinogensis (From Nebert et al. 1993; reproduced by permission).

toxicity. Until a more direct linkage between CYP1A induction and detrimental effects in fish is established, EROD activity is best viewed as an indicator of contaminant exposure rather than of effect. As more information is gathered on the relationship between EROD activity and detrimental effects in fish, this biomarker may also serve as a predictive tool for contaminant risk assessment. In the meantime, EROD induction serves as an economical assessment tool for environmental managers, allowing them to prioritize areas for examination using more expensive techniques such as congener specific analysis of tissue and sediment.

By employing the teleost EROD induction technique with other methods (e.g. H4IIE bioassay, instrumental analysis), an integrated assessment of the contaminant levels and identities in fish will be provided. This multilevel approach renders the best balance of information at the minimal cost.