Use of cytochrome P450 1A (CYP1A) in fish as a biomarker of aquatic pollution

Anders Goksøyr
Laboratory of Marine Molecular Biology, University of Bergen, HIB, N-5020 Bergen, Norway

Development of diagnostic and predictive biomarkers for use in pollution monitoring depends on a fundamental characterization and understanding of the mechanisms and regulations of the molecular response. Since first proposed as a pollution indicator in the mid-1970s (Payne & Penrose, 1975; Ahokas et al., 1975; Payne, 1976), the insight in the cytochrome P450 (CYP) system in fish, its molecular basis, regulation, and the mechanisms underlying the induction response to organic xenobiotics, has increased tremendously. Although the picture is still far from complete, we now have the knowledge and experience to put its potential as a pollution biomarker into use. However, understanding the influences of factors such as water temperature, season, sexual maturation, developmental status, and diet, is an important and critical contribution to the full implementation of CYP1A responses in fish as a biomarker of aquatic pollution.

The cytochrome P450 system

The cytochrome P450 (CYP or P450) system comprise a large superfamily of heme proteins involved in the oxidative metabolism of numerous lipophilic exogenous and endogenous substrates, including drugs, aromatic hydrocarbons, pesticides, fatty acids, prostaglandins and steroids. It is found in all phyla studied to date. The known membership of this superfamily is steadily increasing, with the latest score numbering 221 genes divided into 36 subfamilies based on sequence similarities (Nelson et al., 1993). It is estimated that as many as 60 and perhaps up to 200 different P450 genes are present within a given (mammalian) species (Puga and Nebert, 1989). Due to its importance in pharmacology and carcinogenesis, the major part of the research efforts into this enzyme system is being performed on laboratory rodents and humans. A number of review chapters on this system have been compiled in the recent monograph by Schenkman & Greim (1993).

Although the fundamental features of the monooxygenase reactions are the same in all P450-mediated biotransformations, large differences in the chemistry of both substrate and product exist, from large, complex and bulky molecules to small and planar ones. Different isozymes, belonging to different subfamilies, appear to have varying degrees of substrate specificities, but with some subfamilies dominating in the biotransformation of xenobiotics (Table 1), whereas others are more specifically metabolizing endogenous substrates. Large differences also exist in the regulatory mechanisms of the different isoenzyme forms, ranging from specific cytosolic receptors regulating gene transcription, to posttranscriptional and, in some cases, posttranslational control (Nebert et al., 1989). Certain subfamilies are particularly responsive to xenobiotic compounds, prominent examples being the induction of the CYP1A subfamily by planar aromatic and chlorinated hydrocarbons, and the response of the CYP2B subfamily to the bulky phenobarbital drugs and non-planar PCBs (Table 1). The former response has been observed in all vertebrate species studied, whereas the latter is not detected in fish, amphibia or reptiles (Nebert et al., 1989; Stegeman and Hahn, 1994).
Regulation of CYPIA via the Ah receptor

It is becoming increasingly clear that the CYPIA subfamily is the most important subfamily in the metabolic activation of chemical carcinogens, in that it always directs metabolism towards the formation of reactive, genotoxic intermediates (Ioannides, 1990). Because of this role, the CYPIA subfamily is among the best characterized and well studied in the whole P450 superfamily.

Extensive research has established that planar aromatic hydrocarbons like 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), benzo(a)pyrene (BaP), and certain non- and mono-ortho substituted polychlorinated biphenyls (PCBs), are able to induce the transcription of the CYPIA genes through the aryl hydrocarbon (Ah) receptor. This receptor has recently been shown to belong to a new class of nuclear receptors, distinct from the steroid and thyroid hormone receptor super-family.

Studies have identified a class of response elements, xenobiotic response elements (XREs), upstream of the CYPIA gene through which aryl hydrocarbons activate transcription of the gene. Subsequent to binding a hydrocarbon ligand in the cytosol, the Ah receptor forms a heterodimer with the Ah receptor nuclear translocator (ARNT) protein. This ligand-receptor-translocator complex enters the nucleus where it interacts specifically with the XREs, initiating the transcription of, among others, the CYPIA genes, as depicted in Fig. 1.

In all mammals studied to date, two distinct CYPIA genes, resulting in two different protein products, CYPIA1 and 1A2, are known to exist. The two genes, although both under control of the Ah receptor, are apparently independently regulated by different xenobiotic inducers (CYPIA2 lack XREs), but the mechanisms behind this phenomenon have not been clarified (see chapters in Schenkman & Greim, 1993).

Table 1. Prominent inducible cytochrome P450 subfamilies and their most common substrates

<table>
<thead>
<tr>
<th>Subfamily</th>
<th>Prominent inducers</th>
<th>Common substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYPIA</td>
<td>PAH, BNF, planar PCBs, dioxins</td>
<td>PAH, ethoxyresorufin</td>
</tr>
<tr>
<td>CYPIB</td>
<td>Barbiturates, non-planar PCBs, DDT</td>
<td>Barbiturates, steroids</td>
</tr>
<tr>
<td>CYPIE</td>
<td>Ethanol, ketones</td>
<td>Ethanol, alkylnitrosamines</td>
</tr>
<tr>
<td>CYPIA</td>
<td>PCN, glucocorticoids</td>
<td>Steroids</td>
</tr>
<tr>
<td>CYPIA</td>
<td>Clofibrate, phthalates, 2,4,5-T</td>
<td>Fatty acids</td>
</tr>
</tbody>
</table>

a) Based on Stegeman and Hahn (1994). Abbreviations used: PAH, polyaromatic hydrocarbons; BNF, β-naphthoflavone; PCB, polychlorinated biphenyl; DDT, dichlorodiphenyltrichloroethane; 2,4,5-T, 2,4,5-trichloro?

CYP1A in fish

The duplication event leading to the divergence of mammalian CYPIA1 and CYPIA2 from a single ancestral CYPIA gene is suggested to have occurred less than 250 million years ago (Nebert and Gonzalez, 1987), subsequent to divergence of the fish and mammalian lines of evolution. Whether multiple CYPIA genes occur in fish has been a matter of discussion since the first P450 proteins were purified from fish.

Williams and Buhler (1982; 1984) purified two similar proteins from BNF-treated rainbow trout (Oncorhynchus mykiss) with high activity towards benzo(a)pyrene (P450LM4a and P450LM4b). P450LM4b has later been confirmed to belong to the CYPIA subfamily, based on catalytic properties, inducibility with aromatic hydrocarbons, and immunological relationships (see Stegeman and Hahn, 1994). A single CYPIA-like protein was obtained from scup (Stenotomus chrysops) in John Stegeman’s laboratory (Klotz et al., 1983), and also from cod (Gadus morhua).