Induction of P450IA1 in the livers and gonads of juvenile chinook

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Introduction

Although large amounts of pollutants are being released into aquatic environments, there is considerable lack of knowledge of the mode of action of these compounds in biological systems. Detecting and evaluating the biological changes that result from exposure to pollution are essential steps in detecting the significance of such exposure, and could identify the active pollutant compounds and their source. The ultimate effects of xenobiotics are often seen at the population and ecosystem level, however, these changes originate with biochemical and molecular alterations in individual organisms. The strategy of detecting changes at the molecular level offers distinct advantages as biomarkers; they are usually the first detectable, quantifiable responses to environmental change, they serve as markers of both exposure and effect and they are often more sensitive indicators than changes at higher levels of biological organisation.

Fish incorporate pollutants either directly from the water and sediments or indirectly through the food chain. The incorporation of certain types of xenobiotics, such as some polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), insecticides, pesticides and dioxin causes a rapid increase in the synthesis of enzymes, collectively designated cytochrome P450, which are components of the monooxygenase system. P450IA1 is among the best studied of the P450s, and is the principal one induced in fish exposed to organic pollutants. The enzymes of this system decrease the lipid solubility of organic contaminants, thereby facilitating their excretion.

The induction of P450IA1 in fish can be evaluated by measuring increases in catalytic activity, protein detected immunochemically and mRNA usually detected with cDNA probes. Presently, the most commonly used assays of P450IA1 induction measure increases in catalytic activity (as assessed by EROD and AHH assays). Although undoubtably useful, these enzyme assays do have a number of important limitations. Catalytic activity can be inhibited by some pollutants, especially some organochlorines and heavy metals such as cadmium. Endogenous factors, such as sex steroids, are also known to influence P450IA1 enzyme activity. In addition, these enzyme assays have limited sensitivity and require significant amounts of tissue.

In this study, a new molecular assay was developed to measure P450IA1 mRNA levels in fish exposed to xenobiotics. The assay utilizes reverse-transcriptase - competitive-PCR (RT-cPCR) and has many advantages, particularly its high sensitivity.

Methods and Materials

Fish

Juvenile chinook *(Oncorhynchus tshawytscha)* were used in this series of experiments. Preliminary experiments, conducted between March and August 1993, used chinook maintained in well water (10°C $\pm$ 0.5°C). In September 1993 the stock of juvenile chinook were transferred to sea water (8.7°C $\pm$ 1.3°C), henceforth experimental fish were obtained from this stock of seawater-adapted fish.

Induction method

The juvenile chinook were injected with 50mg\Kg ß-naphthoflavone (ß-NF) body weight i.p. dissolved, by sonication, in corn oil. Control fish were injected with corn oil only. Samples were taken at weekly intervals.

RNA isolation and RT-PCR

Immediately following dissection, pieces of liver and gonads were homogenised in the presence of guanidine isothiocyanate on ice. Total RNA was isolated using the single-step method of Chomczynski and Sacchi (1987). The mRNA for P450IA1 was converted to cDNA in a 20μl reaction volume containing first strand buffer (50mM Tris (pH8.3), 40mM KCl, 1mM DTT, 6mM $MgCl_2$), "Superscript" reverse-transcriptase enzyme (200u; Gibco BRL) and an oligonucleotide "downstream" primer P450IA1-3 (10pmol) designed to anneal to a highly conserved region of the P450IA1 sequence. The cDNA produced was then amplified by polymerase chain reaction (PCR) after the addition of PCR buffer (50mM KCl, 10mM Tris (pH8.3), 100μg\ml gelatin; Gibco BRL), $MgCl_2$ 1.5mM (Gibco BRL), dNTPs (2μM), upstream primer IA1-1 (10pmol), downstream primer IA1-3 (10pmol) and Taq DNA polymerase (0.05u\$\mu$l). Reactions were run for 30 cycles with a 65°C annealing cycle (1.0min), 72°C extension cycle (2.0min), and a 95°C denaturing cycle (1.0min) in a Perkin Elmer Cetus DNA thermal cycler. Results were then be visualised by gel electrophoresis.

This assay can be adapted to be quantitative using a competitive technique. Competitive PCR techniques involve co-amplification in the same tube of two different templates of equal or similar lengths and with the same primer recognition sequences, thus ensuring virtually identical thermodynamics and amplification efficiency for both template species. The different templates compete for amplification and hence, any variable affecting amplification has the same effect on both. As a consequence, the ratio of the PCR products reflects the ratio between the initial amount of the two sequences, thus allowing precise evaluation of the amount of "wild-type" (P450IA1 mRNA) template. The competitive template for our assay was produced using the method of Celi *et al.*, (1993). With this approach, two products are generated by the PCR, one 270 base pair fragment derived from the P450IA1 mRNA isolated from the tissue samples, and another, 120 base pairs smaller derived from the internal competitor. These two fragments are easily resolved by gel electrophoresis.

Results and Discussion

P450IA1 mRNA levels were significantly elevated in the livers of ßNF-treated fish one week following injection. The hepatic P450IA1 mRNA levels continued to increase for a period of five weeks following injection. In addition, P450IA1 was induced in the testes and ovaries of fish treated with ßNF. This gonadal induction was evident one week following injection and P450IA1 mRNA levels continued to accummulate in the gonads for 5 weeks following injection. No P450IA1 induction was detected in the gonads of fish treated with corn oil only. The RT-PCR assay is approximately 1000x more sensitive than the enzyme assays currently being used to assess P450IA1 induction.

The possible functional significance of gonadal P450IA1 induction in fish treated with xenobiotics remains to be elucidated and requires further study. In this regard, it is interesting to note that enzymes of the P450IA family have been shown to metabolise sex steroids in mammals. This study indicates that the measurement of P450IA1 mRNA in various tissues of juvenile fish using RT-cPCR could serve as a sensitive and quantifiable bioindicator of environmental pollution.

References

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Chomczynski, P., and Sacchi, N. (1987). Anal. Biochem. 162: 156-159.