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EROD activity and cytochrome P4501A induction in liver and gills of Senegal sole *Solea senegalensis* from a polluted Huelva Estuary (SW Spain)

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ABSTRACT

EROD activity and induction cytochrome P4501A in liver and gills of Senegal sole, *Solea senegalensis*, from a heavy metal and PAH polluted estuary, was studied. Liver and gill CYP1A catalytic activity was assessed at the enzyme activity level-measured as 7-ethoxyresorufin-*O*-deethylase and cellular localization of CYP1A in the liver was studied using immunohistochemistry. Liver EROD was correlated with phenanthrene-type metabolites in liver and copper concentrations in water. Strong CYP1A occurrence was observed in acinar pancreatic cells, pancreatic duct epithelium and vascular system endothelium and negative/rare induction were observed in hepatocytes and sinusoidal endothelium. In gills, EROD activity showed a significant correlation with different fractions of heavy metals in sediment but no correlation was observed between EROD activity and PAHs. Strongly positive CYP1A associated staining of the vascular system endothelia and primary filament cells and a moderate staining of pillar cells in gills were observed. The results substantiated the utility of EROD activity and CYP1A induction measurement as biomarkers for use by aquatic toxicologists and indicate that catalytic assays and immunohistochemical assays appear to be sensitive to different kinds of pollutants being the use of both methods recommended for monitoring programs.

1. Introduction

Benthic fish from industrialized coastal regions are commonly exposed to long-term stress arising from exposure to sublethal contaminant concentrations. The application of cellular and molecular biomarkers in ecotoxicology depends upon basic information on xenobiotic biotransformation mechanisms.

Cytochrome P450s (CYP) comprises a superfamily of related hemoproteins that, in conjunction with several other enzymes, serve as an electron transport system to catalyze a multitude of monooxygenase reactions. The cytochrome P450 system is responsible for the metabolism of a wide range of endogenous and xenobiotic compounds. It is involved in the transformation of steroids, prostaglandins, fatty acids, and other biological molecules. The CYP system also plays important roles in the toxicology, metabolism, and excretion of pollutants, drugs, and many carcinogenic chemicals and mediates the transformation of certain xenobiotics to their reactive intermediates.

In studies carried out in fish species, CYP1A seem to be a very sensitive biomarker of exposure to organic and inorganic pollutants, which will certainly be feasible in environmental risk assessment (ERA) procedures. CYP1A determinations may be used in various steps of the ERA process, such as quantification of impact and exposure of pollutants, environmental monitoring of organism and ecosystem 'health', identifying subtle early toxic effects, triggering of regulatory action, identification of exposure to specific compounds, toxicological screening and research on toxic mechanisms of xenobiotics. The CYP1A response has been validated for use in ERA monitoring programs (Bucheli and Fent, 1995; Van der Oost et al., 2003), for all these reasons; analysis of CYP1A has been carried out in the present work.

CYP1A is dependent on mixed-function oxygenase (MFO) or monooxygenases. MFO enzyme assays include the ethoxyresorufin-O-deethylase (EROD). MFOs are a family of inducible enzymes which oxidize, by single oxygen addition, natural and anthropogenic chemicals. Their metabolic function assists in the excretion of nonpolar compounds

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(Martin et al., 1985). A number of chemical classes are known inducing agents. The most potent inducers are lipid-soluble, planar compounds of 3 A × 10 A size.

Numerous studies have demonstrated an increase in the CYP1A protein levels in various species of fish after exposure to organic pollutants. Notably, the action of PAHs, PCBs, PCDDs and PCDFs per se caused a significant or a very strong increase (500% of control) in CYP1A content (Van der Oost et al., 2003). Although the main function of the cytochrome P450-dependent monooxygenase system is to convert relatively insoluble organic compounds to soluble metabolites, the resulting metabolites may be less or more toxic than the parent compound activation of cytochrome P450 and it can also promote the CYP1A detoxifying activity. Especially, cytochrome P4501A-dependent oxidation is found to be responsible for the activation of PAHs and PCBs to the reactive intermediates that ultimately result in toxicity, carcinogenicity and mutagenicity (Arinç and Sen, 1999).

Increased EROD activity is frequently observed in fish captured from contaminated waters (Lima et al., 2008; Pathiratne et al., 2008; Lu et al., 2010). EROD levels in fish are usually highest near the contaminant of effluent source, and decreased at sites farther from the source. EROD or AHH activity has been measured in a wide variety of fish species; however, several species show little or no EROD induction (Kloepper-Sams and Benton, 1994; Almroth et al., 2008) or show an inhibition of EROD activity (Mondon et al., 2001; Solé et al., 2006).

Detailed light and microscopic studies of fish liver indicate that the morphology of the liver includes at least 10 resident cell types of CYP1A. By far the most numerous, hepatocytes occupy about 80–85% of the liver volume. Other cell types include endothelial cells, biliary epithelial cells of preductules, ductules and intrahepatic ducts, exocrine pancreatic cells, and centroacinar and ductular cells of exocrine pancreas have shown CYP1A immunoreactivity when fish have been exposed to different contaminants (Desantis et al., 2005; Ortiz-Delgado et al., 2005). The type of inducing agent, dose, species variations, water temperature, reproductive stage and sex may affect the distribution of constitutive and inducible cytochrome P450 forms. Induction responses in endocrine organs may affect important and delicate functions (Husoy et al., 1994).

Vertebrate animals from environments free of pollutant inducers show little or no detectable CYP1A in gills, while animals from contaminated sites express elevated levels of CYP1A (Jönsson et al., 2004; Jimenez-Tenorio et al., 2008). EROD activity in fish liver has been widely studied and considered a good pollution biomarker but CYP1A activity in gills has received less attention (Costa et al., 2011; Nogueira et al., 2011).

The estuary of "Ria de Huelva" has been world-famous as one of the most contaminated estuaries by heavy metals in the world. The contaminant load transported by the Tinto and Odiel rivers to the Ría de Huelva and industrial activities (petrochemical, refining, fertilizer and mining) are the main causes (Sainz et al., 2005). Related to the first contribution, the contaminants come from upstream of Tinto and Odiel rivers where there are mines belonging to the Iberian Pyrite Belt (IPB), an important metal-rich sulfide deposit (Ruiz Cánovas et al., 2012; Galván et al., 2013), and which represent more than 99% of the total metal content in the estuary (Pérez-López et al., 2011).

The commercially exploited Senegal sole, *Solea senegalensis* (Kaup, 1858), represents a suitable bioindicator species for assessment of Huelva estuary pollution. Predominantly littoral, *S. senegalensis* is a benthic marine species well adapted to warm climates and is commonly exploited in extensive aquaculture production in Spain and Portugal (Drake et al., 1984; Dinis, 1992) and has been used in field and laboratory toxicity assays being a sensitive specie to pollutants (Costa et al., 2009; Oliva et al., 2009).

The aim of this work was to study the effect of pollution on the CYP1A cellular induction and EROD activity in liver and gills of *S. senegalensis* and the correlations with heavy metal and PAHs. Results from the current study can also be used to evaluate whether any one measure of CYP1A is more suited for use in monitoring programs.

2. Material and methods

2.1. Sampling sites and fish collection

The Tinto and Odiel Rivers flow together to the Atlantic Ocean forming a common canal known as Canal Padre Santo belonging to the Ria de Huelva.

Three sampling sites were selected in this area of the southwest coast of Spain (Fig. 1): Odiel River (37° 13.5′N 6° 57.2′W), Tinto River (37° 12.5′N 6° 56′W) and Canal del Padre Santo (37° 09.9′N 6° 54.8′W).

Four samplings were realized from October 2004 to May 2006, two of them were realized in autumn (October 2004 and October 2005) and the others were realized in spring (April 2005 and May 2006).

Specimens of Senegal sole *S. senegalensis* (Kaup, 1858) were collected at each sampling site for each sampling period and were transported in aerated tanks to Mazagon's port (Huelva). A total of 97 (mass: 125.04 ± 27.12 g, length: 23.14 ± 1.8 cm) fish were dissected (Odiel River n = 43; Tinto River n = 32, Canal Padre Santo n = 20) and samples of liver and gills of each fish were taken. Fish tissue samples were transported to the laboratory in nitrogen liquid and stored at −80 °C. Twelve specimens of *S. senegalensis* (mass: 182.52 ± 23.89 g, length 25.27 ± 0.78 cm) used as unpolluted or control fish, were obtained from the aquaculture facilities of the Faculty of Marine and Environmental Sciences (University of Cadiz, Spain).

2.2. Heavy metal analysis in fish, sediments and water

Collected fish were dissected and tissue samples from liver and gills were taken. Freeze-dried samples of liver and gills were acid digested by microwave heating using HNO_3 and H_2O_2. Metal concentrations of digested tissue samples were analyzed by ICP-MS and ICP-AES. The accuracy of methodology applied was satisfactorily evaluated using DOLT-3 (dogfish liver) and DORM-2 (dogfish muscle) certified reference materials (Vicente Martorell et al., 2009).

Surface sediment samples (2–20 cm) were collected using a crab and stored in polyethylene bags at −4 °C. Fine particle-size fractions

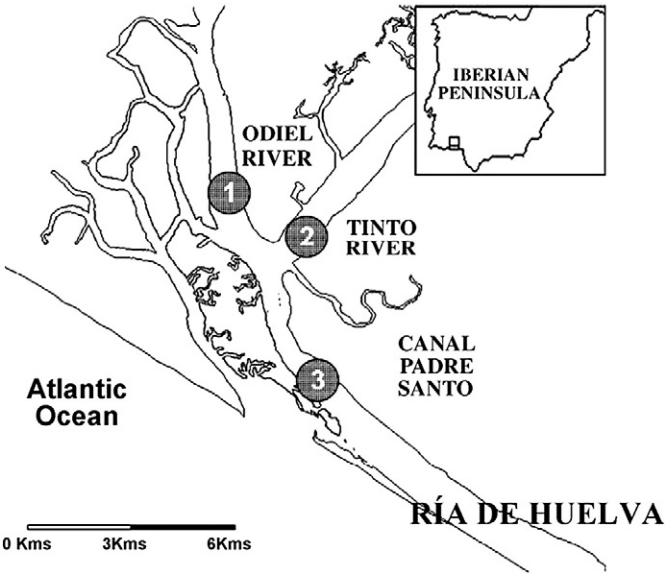

Fig. 1. Location map of sampling sites in Huelva (Spain): 1 (Odiel river); 2 (Tinto river); 3 (Canal Padre Santo).