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Effects of metals and detergents on biotransformation and detoxification enzymes of leaping mullet (*Liza saliens*)

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Abstract

In this study, feral leaping mullet (*Liza saliens*) liver microsomal 7-ethoxyresorufin *O*-deethylase (EROD), and cytosolic glutathione *S*-transferases (GSTs) activities were investigated using 7-ethoxyresorufin, 1-chloro-2,4-dinitrobenzene (CDNB), and ethacrynic acid (EA) as substrates, respectively. The average EROD activity was found as 1139 ± 175 pmol resorufin/min/mg protein. The average GST activities towards CDNB and EA were found as 1364 ± 41 and 140 ± 19 nmol/min/mg protein, respectively. We have, then, investigated the in vitro effects of some metals and detergents on CYP1A and GST activities in leaping mullet liver. Leaping mullet liver microsomal EROD activity was significantly inhibited by Hg (0.1 mM), Ni (0.1 mM), Cd (0.1 mM), Cu (0.1 mM), Zn (0.1 mM), Sb (0.1 mM), Fe^{2+} (1 mM), Co (1 mM), Al (1 mM), and Fe^{3+} (1 mM), with the percent inhibition of 80, 80, 77, 75, 70, 69, 56, 53, 46, and 44, respectively. Similarly, conjugation of CDNB catalyzed by GST was inhibited significantly to lesser extend by Hg (0.1 mM), Sb (0.1 mM), Cd (0.1 mM), Cu (0.1 mM), Zn (0.1 mM), Fe^{3+} (1 mM), Co (1 mM), and Fe^{2+} (1 mM), with the percent inhibition of 70, 69, 65, 61, 54, 51, 47, and 43, respectively. The degrees of inhibition observed on GST catalyzed EA conjugation by Hg (0.1 mM), Cd (0.1 mM), Sb (0.1 mM), Cu (0.1 mM), and Zn (0.1 mM) were 86, 78, 69, 51, and 42, respectively. In addition to metals, the effect of various detergents on leaping mullet liver EROD, GST-CDNB, and GST-EA activities were studied. It was found that ionic detergents strongly inhibited the EROD activity, whereas much less inhibitions were observed with GST catalyzed activities. Therefore, the CYP1A inhibition potencies of metals and detergents suggest that their contribution to the overall CYP1A induction in polycyclic aromatic hydrocarbons contaminated environmental samples has to be taken into account for better interpretation of environmental studies.
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1. Introduction

The aquatic environment is particularly vulnerable to the toxic effects of pollutants, since a considerable amount of the chemicals used in industry, agriculture, and urbanization enter it. These chemicals may affect the biotransformation and detoxification enzymes of aquatic organisms, either as inhibitors or as modulators of enzyme activity. The study of biotransformation enzymes in fish is important in many respects, including evolutionary, ecological, and toxicological standpoints (Moyle and Cech, 1988).

Biotransformation systems are generally regarded as consisting of two subsystems: phases I and II. In phase I metabolism, most lipophilic compounds are oxidized by the cytochrome P450-dependent monooxygenase system with the introduction of hydroxyl groups, yielding a suitable substrate for the phase II reactions. In the phase II subsystem, the metabolite may be conjugated with various polar endogenous substrates, such as glutathione, glucuronic acid, and sulfate, by the transferases to form more soluble products (Benson and Di Giulio, 1992). The phase I metabolizing enzymes have been suggested as biomarkers in hazard assessment of contaminants by a number of authors conducting laboratory or field studies (Fenet et al., 1998; Arinc et al., 2000; Whyte et al., 2000; van der Oost et al., 2003; Sen and Kirikbakan, 2004).

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Cytochrome P450, the terminal oxidase of monooxygenases, is localized mainly in the endoplasmic reticulum and mitochondria of liver and other tissues in fish and other organisms. One of the most widely studied CYP isozymes is CYP1A1 because it is involved in the metabolism of large numbers of cytotoxic, carcinogenic, and mutagenic xenobiotics. CYP1A-associated enzyme activity has been determined by 7-ethoxyresorufin as a substrate. The measurement of 7-ethoxyresorufin *O*-deethylase (EROD) activity appears to be the most sensitive and the most widely used catalytic probe for determining the induction response of CYP1A in fish (Arinc et al., 2000). It is used as a biomarker of exposure to polycyclic aromatic hydrocarbons (PAH) and structurally related compounds (Stegeman and Hahn, 1994; Bucheli and Fent, 1995). However, it has been shown that fish liver microsomal EROD activity may be inhibited by heavy metals (Viarengo et al., 1997; Oliveira et al., 2004) and non-ionic detergents (Yun et al., 1996; Hosea and Guengerich, 1998).

Glutathione *S*-transferases (GSTs) are a group of phase II enzymes and are predominant cytosolic defense systems responsible for protecting cellular components against various toxic effects and oxidative stress (Reed, 1990). Their expression levels in many species can be significantly increased by exposure to foreign compounds, suggesting that they form part of an adaptive response to chemical stress, and they are effective biomarkers of aquatic contamination (Hayes and Pulford, 1995; Sen and Kirikbakan, 2004).

Agricultural, industrial, and household practices have led to the contamination of terrestrial and aquatic environments with a range of inorganic and organic xenobiotics. Among the chemicals involved, heavy metals, and detergents have been extensively used for many years and are, therefore, widely dispersed in the aquatic environment. The aim of the present research was to elucidate in vitro effects of heavy metals and detergents on GST and CYP1A-dependent EROD activities in leaping mullet liver. The metals studied are not only among the environmental toxic pollutants (they represent the most hazardous forms present in the environment) but also the ones widely used in research.

2. Materials and methods

2.1. Chemicals

1-Chloro-2,4-dinitrobenzene (CDNB), ethacrynic acid (EA), glutathione reduced (GSH), ethoxyresorufin, butylated hydroxytoluene (BHT), cholic acid, bovine serum albumin (BSA), β-nicotinamide adenine dinucleotide phosphate (β-NADPH), and phenylmethanesulfonyl fluoride (PMSF) were purchased from Sigma (USA). Ethylenediaminetetraacetic acid disodium salt (EDTA), glycerol, and detergents (Brij 35, CHAPS, Cholate, Deoxycholate, Lubrol, SDS, Triton X-100, Tween 20) were from E. Merck (Germany). Metals ($AlCl_2$, $SbCl_3$, $BaCl_2 \cdot 2H_2O$, $CdCl_2 \cdot H_2O$, $CoCl_2 \cdot 6H_2O$, $CuCl_2$, $FeCl_2 \cdot 4H_2O$, $FeCl_3 \cdot 6H_2O$, KCl, LiCl, $MnCl_2 \cdot 2H_2O$, $HgCl_2$, $NiCl_2 \cdot 6H_2O$, NaCl, and $ZnCl_2$) were purchased from Fluka Chemie (Switzerland). Emulgen 913 was provided by Kao-Atlas Co. Ltd., Tokyo, Japan. All other chemicals were of the highest grade commercially available.

2.2. Fish

Leaping mullet (*Liza saliens*), belongs to the family of Mugilidae of the class Osteichthyes. It is an economically important protein source and is used locally for bioindication of organic pollution in Aegean Sea (Arinc and Sen, 1999; Sen and Kirikbakan, 2004). Leaping mullet, each weighing 300–600 g, were caught by fish net from Pasaport site which is shown to be highly polluted region of Izmir Bay. Izmir is located in the Eastern part of the Aegean Sea of the Mediterranean and is one of the heavily contaminated areas in this part of Turkey.

2.3. Preparation of cytosolic and microsomal fractions of livers from Liza saliens

Fish were killed by decapitation and dissected; livers were then immediately frozen in liquid nitrogen. Tissues were transported in liquid nitrogen from sampling sites to laboratories in Pamukkale University, Denizli. Microsomes were prepared as described by Schenkman and Cinti (1978) and as optimized by Sen and Kirikbakan (2004). Frozen tissues were first thawed on ice and then weighed and minced with scissors. The resulting tissue mince was homogenized in five volumes of 1.15% KCl solution containing 2 mM EDTA, 0.15 mM PMSF, 0.10 mM BHT, and 0.025% cholate by using Potter-Elvehjem glass homogenizer (packed in crushed ice) coupled with motor (Black & Decker Skill Plus, multispeed drill)-driven Teflon pestle at 2600 rpm, ten passes were made for homogenization. The homogenate was then centrifuged at 12,000 *g* by using Hermle Z323 K high-speed refrigerated centrifuge (220.80.V03 rotor, Hermle Labortechnik GmbH Gosheimer Str. 56 D-78564 Wehingen, Germany) for 25 min. Aliquots were removed and remaining fractions were filtered through doubled layers of cheese cloth. Then, 0.4 volumes of 0.16 mM $CaCl_2$ was added to the supernatant fractions and re-centrifuged at 20,000*g* for 75 min. Calculated volumes of ice-cold glycerol was added as a stabilizer to the resulting supernatant (cytosol) fractions to obtain 10% final concentration, and pellet (microsomes) were suspended in homogenization solution and re-centrifuged as above. The resulting washed microsomal pellet was resuspended in 10% glycerol containing 2 mM EDTA and homogenized manually using glass-Teflon homogenizer. All fractions were stored at $-80\,^{\circ}C$ until use.

2.4. Measurements of protein concentration

Protein concentrations of the cytosolic and microsomal fractions of livers from *L. saliens* were determined by the Lowry method (Lowry et al., 1951) using crystalline BSA as standard.

2.5. Enzyme assays

EROD activities of microsomes were determined by spectrofluorometric method of Burke and Mayer (1974) with some modifications (Sen and Arinc, 2000). The reaction mixture contained 0.1 M potassium phosphate buffer, pH 7.8, 0.1 M NaCl, 1.2 mg BSA, 50–100 μg of fish liver microsomal protein, 0.1 mM NADPH in the presence of substrate, 1.5 μM 7-ethoxyresorufin. The reaction was initiated by the addition of substrate and the progressive increase in fluorescence, resulting from resorufin formation, was measured during 2 min (excitation wavelength 535 nm, emission wavelength 585 nm).

GST activities using CDNB (measured in 0.1 M potassium phosphate buffer, pH 7.5 containing 1 mM CDNB, 1 mM GSH, 25 μg cytosolic protein), and EA (carried out in 0.1 M Tris–HCl, pH 7.8 containing 0.2 mM EA, 0.5 mM GSH, 25 μg cytosolic protein) as substrates were determined at room temperature spectrophotometrically by following the change in absorbencies at 340 and 270 nm, respectively (Habig et al., 1974). Effects of metals and detergents on enzyme activities were

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