ELSEVIER

Aquatic Toxicology 79 (2006) 24–30

AQUATIC TOXICOLOGY

www.elsevier.com/locate/aquatox

Sulfotransferase activities towards xenobiotics and estradiol in two marine fish species (*Mullus barbatus* and *Lepidorhombus boscii*): Characterization and inhibition by endocrine disrupters

Rebeca Martin-Skilton [a], Michael W.H. Coughtrie [b], Cinta Porte [a,*]

[a] *Environmental Chemistry Department, IIQAB-CSIC, C/Jordi Girona 18, 08034 Barcelona, Spain*

[b] *Division of Pathology & Neuroscience, University of Dundee, Ninewells Hospital & Medical School, Dundee DD1 9SY, Scotland, UK*

Received 13 January 2006; received in revised form 25 April 2006; accepted 27 April 2006

Abstract

We have characterized hepatic phenol sulfotransferase (SULT) activities in two benthic fish species, *Mullus barbatus* and *Lepidorhombus boscii*, using *p*-nitrophenol, dopamine, 17β-estradiol, 4-nonylphenol, and 1-naphthol as substrates. High affinity sulfation of 17β-estradiol was observed in both species (K_m = 28–75 nM), suggesting the presence of a specific estrogen sulfotransferase that catalyzes the formation of estradiol-3 sulfate. Among the tested compounds, 1-naphthol was the most effective substrate for sulfation, with V_{max}/K_m ratios several hundred-fold higher than the other substrates examined. Both species sulfated the tested compounds, but only *M. barbatus* was able to sulfate dopamine. We also tested the inhibitory effects of common marine pollutants with estrogenic (4-nonylphenol) and androgenic (tributyltin, triphenyltin) properties on *p*-nitrophenol and 17β-estradiol SULT activities. 4-Nonylphenol and triphenyltin inhibited sulfation of both substrates at micromolar concentrations in both species. However, tributyltin was only effective against SULTs from *L. boscii*, again at micromolar concentrations. The data indicate that *M. barbatus* and *L. boscii* are able to sulfate a range of xenobiotics and endogenous compounds, and inhibition of these activities by environmental pollutants may contribute to the known toxic effects of these compounds.

Keywords: Sulfotransferases; Estradiol; Endocrine disruption; Fish; Nonylphenol; Organotin compounds

1. Introduction

Sulfation plays a critical role modulating the biological activity, and facilitating the elimination, of potent endogenous chemicals including steroids, thyroid hormones, and catechols, and also many synthetic chemicals or xenobiotics (Kauffman, 2004). Sulfation reactions are catalyzed by sulfotransferase (SULT) enzymes which transfer the sulfuryl group from 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to an appropriate group (usually –OH) on the substrate. Like many xenobiotic-metabolizing enzymes, cytosolic sulfotransferases are derived from a large gene superfamily. In humans, representatives of four SULT families do exist (SULT1, SULT2, SULT4 and SULT6) comprising at least 12 different isoforms (Coughtrie, 2002; Freimuth et al., 2004). The SULT1 and SULT2 families are the largest, and are responsible for sulfation of a vast number of endogenous and foreign compounds (Kauffman, 2004).

Full-length cDNAs for more than 60 mammalian and avian species have been cloned and sequenced, and many of the expressed proteins characterized (Coughtrie, 2002; Nagata and Yamazoe, 2000). However, information concerning fish species is rather limited. Hepatic and intestinal phenol sulfotransferases have been studied, using a range of substrates in the channel catfish (*Ictalurus punctatus*) and the mummichog (*Fundulus heteroclitus*) (Tong and James, 2000; Gaworecki et al., 2004). The enzymes catalyzed the sulfation of a variety of xenobiotic and physiologically important compounds, and were especially active towards phenolic substrates. Recently, a number of SULTs from the zebrafish (*Danio rerio*) have been characterized. Sugahara et al. (2003a) studied a zebrafish sulfotransferase belonging to the SULT1 gene family; this sulfotransferase displayed activity towards a number of endogenous compounds, in particular dopamine and thyroid hormones, in addition to xenobiotic compounds including some flavonoids, isoflavonoids, and other phenolic compounds. The SULT2 family, known to cat-

* Corresponding author. Tel.: +34 93 4006175; fax: +34 93 2045904.
E-mail address: cpvqam@cid.csic.es (C. Porte).

0166-445X/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquatox.2006.04.012

alyze the sulfation of 3β-hydroxy groups in steroids with unsaturated A rings, has also been identified in zebrafish (Sugahara et al., 2003b), and this isoform displayed 44%, 43% and 40% amino acid identity with mouse SULT2B1, human SULT2B1 and human SULT2A1, respectively.

There is also evidence that several xenobiotics, by acting as structural mimetics, can inhibit the sulfation of steroids in fish, and disrupt endocrine-mediated events, such as the modulation of steroid hormone activity. This has been demonstrated to be a potential mechanism of endocrine disruption by hydroxylated polychlorinated biphenyls (Kester et al., 2000, 2002). In addition, alkylphenols have a strong inhibitory effect on the sulfation of estrone in the chub (*Leuciscus cephalus*) (Kirk et al., 2003) and tributyltin (TBT), triphenyltin (TPT), and nonylphenol (NP) were inhibitors of the sulfation of 17β-estradiol in carp (*Cyprinus carpio*) cytosolic fractions (Thibaut and Porte, 2004). However, apart from these studies and despite the key role of SULT enzymes on endogenous and xenobiotic metabolism, information on sulfation in aquatic species (marine in particular) is limited. Thus, the aims of this work were to examine hepatic SULT activity in two benthic fish species, the red mullet (*Mullus barbatus*) and the four spotted megrim (*Lepidorhombus boscii*), using *p*-nitrophenol (*p*-NP), dopamine (DA), and 17β-estradiol (E2) as substrates, as they are reputedly "diagnostic" probes for different SULT1 isoforms in humans, namely SULT1A1 (*p*-NP), SULT1A3 (DA), and SULT1E1 (E2) (Dajani et al., 1998; Nagata and Yamazoe, 2000). As these fish are commonly exposed to polycyclic aromatic hydrocarbons, detergents, and many other xenobiotics in coastal areas polluted by shipping and industrial wastes (Porte et al., 2002; Borghi and Porte, 2002), two additional compounds, 1-naphthol and 4-nonylphenol, were comparatively tested as substrates. Finally, the effects of three xenobiotics, NP, TBT and TPT, on the sulfation of *p*-NP and E2 were examined.

2. Materials and methods

2.1. Materials

[6,7-^{3}H]-Estradiol (49.7 Ci/mmol; >97% purity), PAP^{35}S (2.245 Ci/mmol; >97% purity) were purchased from Perkin-Elmer Life Sciences (Cambridge, UK). PAPS (>99% purity) was purchased from H. Glatt, German Institute for Human Nutrition (Potsdam, Germany). Dopamine, *p*-nitrophenol, 17β-estradiol, 1-naphthol, tributyltin, triphenyltin and 4-nonylphenol (97% purity) were purchased from Sigma–Aldrich (Steinheim, Germany). Recombinant human sulfotransferase isozyme 1E was from Sigma (Steinheim, Germany).

2.2. Sample collection

Red mullet (*M. barbatus*) (12–19 cm) were collected along the Mediterranean coast (NE Spain) by bottom trawling at a 30–50 m depth in May 2002. Four-spotted megrim (*L. boscii*) (23–30 cm) were collected from the Galician coast (NW Spain) by bottom trawling at 200 m depth in April 2002. Immediately after collection, fish were sacrificed by severing the spinal cord, the liver dissected, directly frozen in liquid nitrogen, and stored at −80 °C for further analysis.

Both fish species were in the pre-spawning season. Females of *L. boscii* had ovaries with some vitellogenic granules of various sizes and development, with few to moderate numbers of vitelline granules and males were at mid-spermatogenic stage. Only females of *M. barbatus* were available for the study, those females had ovaries with mature oocytes containing numerous densely packed vitelline granules.

2.3. Preparation of cytosolic fractions

After being thawed and weighed, livers were flushed in ice-cold 1.15% KCl, and homogenised (1:5, w/v) in ice-cold 100 mM KH_2PO_4/K_2HPO_4 buffer pH 7.4, containing 150 mM KCl, 1 mM EDTA, 1 mM dithiothreithol (DTT), and 0.1 mM phenylmethylsulfonyl fluoride. Homogenates were centrifuged at 1500 × *g* for 15 min, the fatty layer removed and the resulting supernatant centrifuged at 12,000 × *g* for 20 min. The 12,000 × *g* supernatant was further centrifuged at 100,000 × *g* for 60 min to obtain the cytosolic fraction (supernatant). Protein content was estimated by the method of Lowry et al. (1951) using bovine serum albumin as standard.

2.4. Enzymatic assays

The PAP^{35}S barium precipitation assay (Foldes and Meek, 1973) was used to measure SULT activity with *p*-NP, DA, 1-naphthol, and NP as substrates. *p*-NP and DA were added from an aqueous solution; 1-naphthol and NP were made in dimethylsulfoxide (DMSO), maximal solvent concentration in the assay was of 1.25% (v/v). Protein, incubation time, and substrate concentrations were optimized for each assay. All assays were carried out in duplicate, plus a control assay, each for three individual fish. Control incubations contained vehicle in place of substrate. Reaction mixtures (final volume 160 μl) comprised 0.1 M potassium phosphate pH 7.4, PAPS (10 μM final concentration, containing 0.054 μCi PAP^{35}S), cytosolic protein (100–150 μg) and substrate (0.05 μM to 2 mM). The reactions were incubated for 20 or 30 min (DA) at 30 °C, and stopped by placing on ice and adding 100 mM barium acetate (200 μl), followed by 100 mM barium hydroxide (200 μl), and 100 mM zinc sulfate (200 μl) causing the precipitation of unreacted PAPS. The samples were centrifuged at 13,000 × *g* for 4 min, and 500 μl of the supernatant were then mixed with 4 ml scintillation cocktail (OptiPhase Hisafe 2, Perkin-Elmer Life Sciences), and the radioactivity was quantified by liquid scintillation counting (Tri-Carb 2100 TR, Packard). Enzyme activities are presented per minute per milligram of cytosolic protein.

To assess the sulfation of E2, reaction mixtures (final volume 200 μl) contained 50 mM Tris–HCl pH 7.5, 10 mM $MgCl_2$, PAPS (20 μM), cytosolic protein (100 μg), and [^{3}H]-estradiol (0.2 nM to 50 μM). The reactions proceeded for 20 min at 30 °C, and were stopped by placing on ice followed by the addition of ice-cold distilled water (300 μl), and the unmetabolized estradiol extracted with 3 ml of methylene chloride (×2). After extraction, an aliquot of 200 μl was quantified by liquid