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Intestinal glutathione S-transferase activity in flounder *Platichthys flesus* collected from contaminated and reference sites

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ABSTRACT: Intestinal glutathione S-transferase activities were measured during the GEEP Workshop in flounder *Platichthys flesus* collected from Langesundfjord, Norway. Though enzyme activities were generally higher in fish collected from 3 polluted sites by comparison with a reference site, the only significant differences in activities between the 3 sites ran counter to the anticipated pollution gradient. The results suggest that intestinal glutathione S-transferase activity may not be a sensitive indicator of pollution exposure under field conditions.

INTRODUCTION

The glutathione S-transferases are a family of enzymes which play an important role in detoxification and elimination of xenobiotics (Jakoby 1978). Within the cell, the cytosolic glutathione S-transferases can aid in the detoxification of xenobiotics in 3 complementary ways: (1) these enzymes may increase the availability of lipophilic toxicants to microsomal cytochromes P-450 by serving as carrier proteins (Hanson-Painton et al. 1983); (2) glutathione S-transferases serve as catalysts for the conjugation of glutathione with electrophiles including those produced during the metabolism of xenobiotics by the cytochrome P-450 system (Nemoto & Gelboin 1975); (3) by covalently binding to electrophilic compounds, glutathione S-transferases can reduce the likelihood of the binding of these compounds to cellular macro-molecules such as DNA (Schelin et al. 1983).

Glutathione S-transferases are widely distributed in hepatic and extra-hepatic tissues including those of marine fishes (James et al. 1979). In mammals, the activity of these enzymes in several organs is sensitive to dietary inducing agents such as polycyclic aromatic hydrocarbons (PAH) and phenobarbital (Clifton & Kaplowitz 1978). In laboratory studies with fish, we have observed enhanced activity of hepatic and intestinal glutathione S-transferases following exposure of fish to a diet containing the PAH benzo[a]pyrene (Van Veld & Lee unpubl.). The GEEP Workshop gave us the opportunity to study further the role of the intestine in the metabolism of dietary toxicants and to investigate the feasibility of using intestinal glutathione S-transferase activity as an indicator of environmental pollution.

Intestinal glutathione S-transferase activity was measured in flounder *Platichthys flesus* collected from reference and polluted sites in the Langesundfjord area of Norway. The nature of the organic contaminant gradient present at the 4 field sites at which flounder were collected is discussed by Follum & Moe (1988) and Klungsøyr et al. (1988); see also Appendix 1. The 4 sites were designated 1 to 4 in increasing order of anticipated contaminant impact.

METHODS

Live *Platichthys flesus* were transported to the laboratory on the morning of capture in Langesundfjord, Norway, as described by Follum & Moe (1988). In sexually mature individuals of several species of estuarine fish, intestinal glutathione S-transferase activity does not vary significantly with sex or age (Van Veld & Lee unpubl.). For the present study, mature individuals (ca 300 to 900 g) of both sexes were used. Body cavities were opened and the first half of the intestine distal to the stomach was removed. Only

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flounder with food visible in the intestine were used. Intestines were flushed with ice-cold Tris-HCl, pH 7.4 (Buffer A) containing 0.1 m*M* phenylmethylsulphonyl fluoride (PMSF) in an ethylene glycol monomethyl ether carrier. Intestines were homogenised using several low-speed bursts with a polytron (Brinkman Instruments) in 3 volumes of Buffer A containing PMSF. Homogenates were centrifuged twice at 12 000 × *g* for 10 min, the pellet and fat pad being discarded after each centrifugation. The 12 000 × *g* supernatant was centrifuged twice at 105 000 × *g* for 1 h, and the pellet and fat layer were removed.

Glutathione S-transferase activity using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate was determined spectrophotometrically (Habig et al. 1974). Reaction mixtures consisted of Buffer A containing 1 m*M* concentrations of reduced glutathione (Sigma Chemical Co., St. Louis, MO, USA), 1 m*M* CDNB and 50 to 100 µg of cytosolic protein per ml. Glutathione S-transferase activity using ^{3}H-benzo[a]pyrene-4,5-oxide (BPO) (Chemsyn Science Laboratories, Lanexa, KA, USA) were performed according to a modification of a procedure outlined by Dostal et al. (1986). Reaction mixtures consisted of Buffer A containing 10 m*M* reduced glutathione, 0.1 m*M* BPO and 250 to 500 µg of cytosolic protein per ml in a total volume of one ml. Controls contained boiled cytosol. After incubation at 37 °C for 5 min, reactions were stopped and the unconverted substrate and any diols formed were removed by 4 extractions with 2.5 ml of ethyl acetate. The radioactivity remaining in the aqueous phase was counted in a liquid scintillation counter for determination of the amount of conjugates formed. Cytosolic protein concentration was determined by the method of Bradford (1976). Data were analysed by ANOVA followed by multiple comparison tests to determine if statistically significant differences in enzyme activities existed between fish collected from different sites.

RESULTS AND DISCUSSION

Results of analysis for intestinal glutathione S-transferase activity towards CDNB and BPO in *Platichthys flesus* are presented in Figs. 1 and 2, respectively. For both substrates, no clear trends in enzyme activities were observed along the expected gradient from reference to contaminated sites (1 to 4). Although activity towards CDNB in samples from the reference site was significantly lower than in samples from Site 2, differences between Sites 1 and 3, and Sites 1 and 4, just failed to attain significance. However, for activity towards BPO, all 3 polluted sites did have significantly higher values than for the reference site, though activity at Site 4 was lower than at the 2 intermediate sites

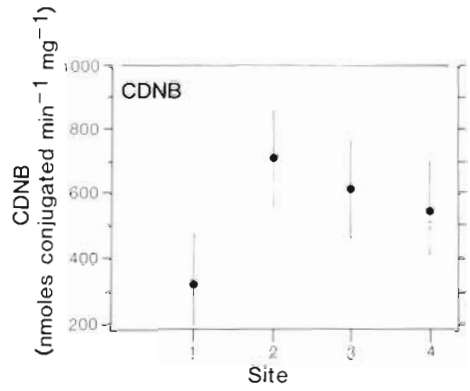

Fig. 1. *Platichthys flesus*. Intestinal glutathione S-transferase activity using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate, for the 4 Langesundfjord sites (mean ± 2 SE, n = 5)

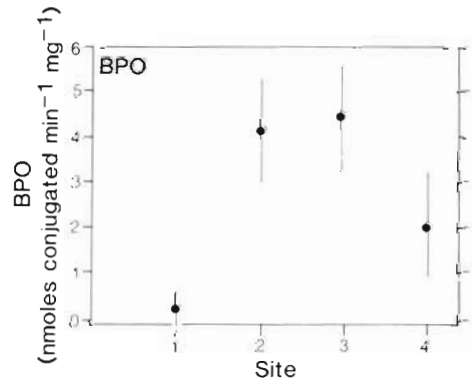

Fig. 2. *Platichthys flesus*. Intestinal glutathione S-transferase activity using ^{3}H-benzo[a]pyrene-4,5-oxide (BPO) as substrate, for the 4 Langesundfjord sites (mean ± 2 SE, n = 5)

(significantly so in comparison with Site 3). For both substrates tested it is possible that the trend towards lower activity in samples from the reference site may have resulted, in part, from the relatively poor (i.e. hypoxic) condition of reference site fish following the transport of these fish to the laboratory; later samples were transported in oxygenated rather than aerated containers and arrived in better condition.

Although intestinal glutathione S-transferases are known to be sensitive to dietary pollutants including polycyclic aromatic hydrocarbons (Clifton & Kaplowitz 1978), results from the present study indicate that intestinal glutathione S-transferase activity may not be a sensitive indicator of pollution exposure in the field. Under controlled laboratory conditions we routinely observe an approximate 2-fold increase in glutathione S-transferase activity in fish intestines following administration of PAH contaminated diets. However, a response of this magnitude may be insufficient to allow detection of differences in enzyme activities under field conditions. While intestinal glutathione S-transferase activity was not a useful indicator of pollution exposure

in the field study, other drug metabolizing enzymes of the intestine may in some cases show promise in studies of this type. Intestinal mono-oxygenase activity is very sensitive to dietary inducing agents. For example, following oral administration of the aromatic hydrocarbon 3-methylcholanthrene to rats, Stohs et al. (1976) observed a 30-fold stimulation in intestinal aryl hydrocarbon hydroxylase activity and a 20-fold increase in 7-ethoxyresorufin O-de-ethylase activity. In our studies we have observed enhanced activity of aryl hydrocarbon hydroxylase (10-fold) and 7-ethoxyresorufin O-de-ethylase (24-fold) in intestinal microsomes prepared from spot *Leiostomus xanhurus* following administration of a PAH-contaminated diet (Van Veld & Lee unpubl.). However, as is commonly encountered in some mammalian species (Wollenberg & Ullrich 1980) we sometimes experience problems in obtaining stable mono-oxygenase activity during the preparation of intestinal microsomes from some species of fish. *Platichthys flesus* proved to be one of these problem species. Clearly, stability is a prerequisite for the use of intestinal mono-oxygenase activity as a potential signal of pollution exposure.

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