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Induction of Cytochrome P450 1A1 and Monooxygenase Activity in Tilapia by Sediment Extract

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Cytochrome P450 (P450)-dependent monooxygenases of fishes are inducible by a variety of environmental pollutants including polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) (Stegeman 1989). Induction of fish monooxygenases may serve as a biological monitor for PAH- and PCB-types of environmental chemicals (Payne et al. 1987; Stegeman and Lech 1991; Goksoyr and Forlin 1992). Many studies have demonstrated environmental induction of fish monooxygenases using various experimental approaches such as pumping contaminated water into fish tanks in the laboratory, suspending cages of fish in polluted water, and comparing monooxygenase activities in fish colected from clean and polluted areas (Kleinow et al. 1987; Payne et al. 1987). However, relatively few studies have been conducted using fish treated with contaminated river sediment extracts.

Damsui River is the largest river in the north of Taiwan. The lower section of the river in the Taipei Metropolitan area is heavily polluted by industrial and municipal wastes. Tilapia (*Oreochromis mossambicus*) is one of the few species of fish that occur in the polluted river. Previous field studies showed that the levels of P450 1A1, benzo(a)pyrene hydroxylase and 7-ethoxyresorufin O-deethylase activities in tilapia collected at Fu-Ho Bridge, a polluted section of Damsui River, were higher than respective levels in fish collected from an unpolluted section (Liu et al. 1991; Ueng et al. 1992). These results suggested that tilapia caught at the polluted site were exposed to substances similar in action to PAHs and PCBs, because these chemical pollutants are potent inducers of P450 1A1 (Ueng et al. 1992).

PAHs and PCBs are persistent compounds that can accumulate in sediment. Tilapia are occasionally associated with the bottom

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and could ingest chemically contaminated sediment. In the present study, we determined the induction properties of monooxygenases using tilapia treated with extract of sediment collected from a polluted section of Damsui River. The present study demonstrates that Damsui River sediment extract has the ability to induce hepatic P450 1A1 and dependent monooxygenase activities in tilapia.

MATERIALS AND METHODS

Male tilapia hybrid (*Oreochromis niloticus* female x *O. aureus* male) weighing 350-400 g were used for the study. Fish were purchased from Ta-Yuan fish farm, Tao-Yuan, Taiwan, R.O.C.. Before the experiments began, animals were acclimated for at least one-month in the animal quarters with air conditioning and automatic 12-hr photocycle. The animals were fed *ad libitum* with a commercial fish diet. An initial study was done to determine the effects of two xenobiotics on tilapia monooxygenases. Fish were pretreated with 3-methylcholanthrene (3-MC) in corn oil at 20 mg/kg, intraperitoneally (i.p.). Aroclor 1254 (PCB), a PCB mixture, was similarly administered at 30 mg/kg. Control fish were treated with corn oil only. The animals were killed 18 hr later. Livers were removed and washed microsomes were prepared.

In the sediment induction study, sediment was collected at Fu-Ho Bridge, a polluted site of Damsui River (Liu et al. 1991). Fifty g of sediment were extracted twice using methanol and dichloromethane following the protocol described by Collier et al. (1986). The sediment extract was dissolved in 5 mL of acetone and Emulphor 620 (1:1) and administered to tilapia at 2.0 mL/kg i.p. The fish were killed 18 hr later. Control fish were treated with the vehicle only.

Microsomal P450 content was determined by the method of Omura and Sato (1964). Benzo(a)pyrene hydroxylase activity was determined by measuring the formation of the phenolic metabolites according to the fluorimetric method of Nebert and Gelboin (1968) with 3-hydroxybenzo(a)pyrene as a standard. 7-Ethoxyresorufin O-deethylase activity was determined by measuring the fluoresence of the deethylated product, resorufin, following the procedures of Pohl and Fouts (1980). Protein concentration was determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using the discontinuous system of Laemmli (1970) as described previously (Ueng et al. 1992). Transfer of microsomal proteins from slab gel to nitrocellulose membrane was carried out following the procedures of Towbin et al. (1979). Immunodetection of P450 1A1 was performed using a mouse monoclonal antibody (MAb) 1-12-3 prepared against scup liver P-450E, a P450 1A1 homologue in fish (Park et al. 1986). The MAb was kindly provided by Dr. Sang S. Park, NCI-FCRDC, Frederick, Maryland, U.S.A.. The statistical significance of differences between control and treated groups was evaluated by the student's *t* test. A p value of less than 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

In the initial study, single doses of 3-MC and PCB caused 17- and 3-fold increases in hepatic microsomal benzo(a)pyrene hydroxylase activity, respectively (Table 1). The 3-MC and PCB treatments resulted in 54- and 14-fold increases of 7-ethoxyresorufin O-deethylase activity, respectively. Hepatic P450 contents in 3-MC- and PCB-treated fish were similar to the controls. These results indicated that 3-MC and PCB induced a specific form of P450, namely 1A1, in tilapia, because benzo(a)pyrene and 7-ethoxyresorufin are the preferential substrates for the cytochrome (Stegeman 1989). To further study this inductive effect, liver microsomes were subjected to SDS-PAGE, followed by blotting and immunochemical detection procedures, in which mouse MAb 1-12-3 raised against scup liver P450 1A1 (Park et al. 1986) was used to probe for immunorelated P450s. A weak immunoreactive protein band was detected at 59,000 molecular weight region in control fish (Fig. 1, lane 1). The intensity of this protein band was markedly increased in fish pretreated with 3-MC and PCB (lanes 2 and 3). These immunochemical results showed that 3-MC and PCB treatments induced P450 1A1 in tilapia, consistent with the results of monooxygenase activity studies (Table 1).

Table 1. Acute effects of 3-methylcholanthrene (3-MC) and Aroclor 1254 (PCB) on microsomal monooxygenases in tilapia liver.[1]

Assay	Control	3-MC	PCB
Cytochrome P450 (nmol/mg protein)	0.25± 0.02	0.29± 0.02	0.25± 0.02
Benzo(a)pyrene hydroxylase (pmol /min/mg protein)	28.5 ± 6.6	471.2 ± 34.3*	98.2 ± 29.0*
7-Ethoxyresorufin O-deethylase (pmol /min/mg protein)	15.0 ± 4.6	808.8 ±103.7*	208.3 ± 47.7*

[1]Tilapia were administered 3-MC and PCB at 20 and 30 mg/kg i.p., respectively. Control fish were treated with corn oil only. The animals were killed 18 hr later. Liver microsomes were prepared and monooxygenases were assayed as described in the Materials and Methods section. Each value represents the mean ± S.E. for at least 5 fish.

*Value significantly different from respective control value, $p < 0.05$.

In the sediment study, treatment of tilapia with sediment extract caused 6- and 7-fold increases in hepatic benzo(a)pyrene hydroxylase and 7-ethoxyresorufin O-deethylase activities, respectively (Table 2). Hepatic P450 content in treated tilapia

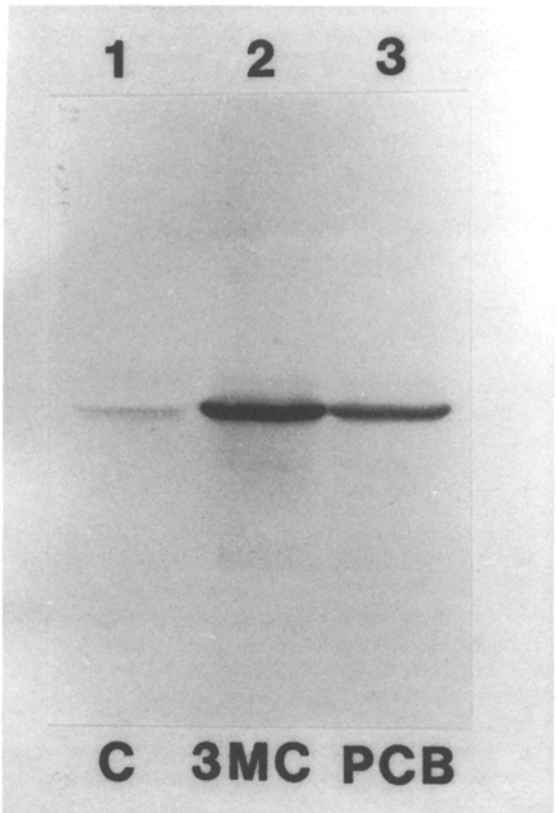

Figure 1. Immunoblot analysis of liver microsomal P450 1A1 from tilapia pretreated with 3-MC and PCB. The fish were pretreated with the inducing agents as described in the legend to Table 1. Microsomal proteins were subjected to SDS-PAGE, transferred to a nitrocellulose membrane, and immunochemically stained with mouse MAb 1-12-3 to scup liver P450 1A1. Lanes 1-3 contained microsomes from control (C), 3-MC- and PCB-treated tilapia as indicated. Thirty ug of protein were applied to each lane.

was similar to the controls. These inductive properties of monooxygenases (Table 2) are similar to the properties observed in tilapia pretreated with 3-MC and PCB (Table 1), showing increases of P450 1A1-dependent monooxygenase activities. To further investigate this inductive effect, liver microsomes from control and sediment-treated tilapia were subjected to electrophoresis and immunoblotting studies using MAb 1-12-3 against scup P450 1A1 (Fig. 2). The MAb showed a weak cross reactivity with a protein band at 59,000 molecular weight region in control fish (lanes 1 and 2). Acute treatment with sediment extract markedly increased the intensity of the protein immunorelated to P450 1A1 (lanes 3 and 4).

In this report, we show concomitant induction of P450 1A1 and dependent monooxygenase activities in tilapia treated with

Table 2. Acute effects of Damsui River sediment extract on microsomal monooxygenases in tilapia liver.[1]

Assay	Control	Sediment Extract
Cytochrome P450 (nmol/mg protein)	0.24± 0.06	0.26± 0.06
Benzo(a)pyrene hydroxylase (pmol /min/mg protein)	20.0 ± 5.4	127.8 ± 8.4*
7-Ethoxyresorufin O-deethylase (pmol /min/mg protein)	24.5 ± 9.9	168.5 ±19.7*

[1]Tilapia were administered i.p. sediment extract at 2.0 mL/kg. The sediment was collected at Fu-Ho Bridge, a polluted site of Damsui River. Control fish were treated with acetone alone. The animals were killed 18 hr later. Liver microsomes were prepared and monooxygenases were assayed as described in the Materials and Methods section.
Each value represents the mean ± S.E. for at least 5 fish.
*Value significantly different from respective control value, $p < 0.05$.

sediment extract. There are, however, other reports in the literature showing induction of either P450 1A1 or monooxygenase activity in sediment-treated fish. For instance, Schoor et al. (1991) reported that P450 1A1 was induced in juvenile guppies chronically exposed to creosote-contaminated sediment collected near Pensacola, Florida, U.S.A.; P450 1A1-dependent monooxygenase activity was not reported. Collier and Varanasi (1991) showed that benzo(a)pyrene hydroxylase activity was increased in English sole pretreated with extract of sediment collected from a contaminated site in Puget Sound, Washington, U.S.A., whereas P450 1A1 was not determined in these fish.

Our results indicate a marked similarity in induction properties of monooxygenases in tilapia pretreated with PAH, PCB, and sediment extract. In previous studies (Liu et al. 1991; Ueng et al. 1992), we reported environmental induction of monooxygenases in tilapia caught at the sediment sampling site. Even though the tilapia collected from Damsui River and the tilapia used in this study were different species, our current and previous findings suggest that the induction of monooxygenases in feral tilapia may have resulted from exposure to substances similar in action to PAH and PCB. The induction of monooxygenases in tilapia may have occurred through ingestion of the contaminated sediment. Our results also show that the degrees of induction of monooxygenase activities in tilapia treated with sediment extract were comparable to the degrees of

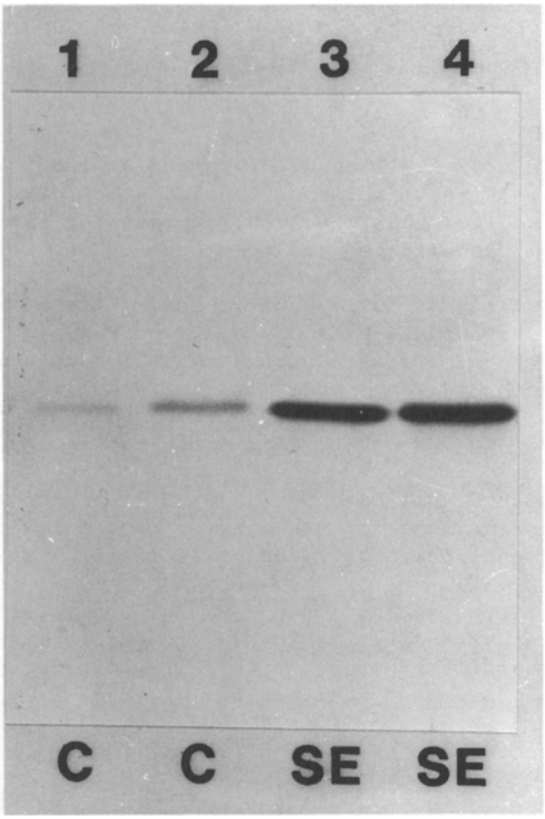

Figure 2. Immunoblot analysis of liver microsomal P450 1A1 from tilapia pretreated with extract of polluted Damsui River sediment. The fish were pretreated with the sediment extract as described in the legend to Table 2. Microsomal proteins were subjected to SDS-PAGE, transferred to a nitrocellulose membrane, and immunochemically stained with mouse MAb 1-12-3 to scup liver P450 1A1. Lanes 1 - 4 contained microsomes from control (C) and sediment extract (SE)-treated fish as indicated. Thirty ug of protein were applied to each lane.

induction in fish treated with PCB (Tables 1 and 2). However, the amounts of PCB or PAH present in the sediment cannot be estimated based on these results, because the sediment extract is a mixture of chemicals which may have the ability to induce or suppress the levels of monooxygenases in the fish.

In conclusion, the present study demonstrates that sediment extract from the polluted Damsui River has the ability to cause a marked induction of tilapia hepatic monooxygenases and that the sediment may be contaminated with PAH- and PCB-types of substances.

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REFERENCES

Collier TK, Stein JE, Wallace RJ, Varanasi U (1986) Xenobiotic metabolizing enzymes in spawning English sole (*Parophrys vetulus*) exposed to organic-solvent extracts of marine sediments from contaminated and reference areas. Comp Biochem Physiol 84C:291-298

Collier TK, Varanasi U (1991) Hepatic activities of xenobiotic metabolizing enzymes and biliary levels of xenobiotics in English sole (*Parophrys vetulus*) exposed to environmental pollutants. Arch Environ Contam Toxicol 20:462-473

Goksoyr A, Forlin L (1992) The cytochrome P-450 system in fish, aquatic toxicology and environmental monitoring. Aquat Toxicol 22:287-312

Kleinow KM, Melancon MJ, Lech JJ (1987) Biotransformation and induction: Implications for toxicity, bioaccumulation and monitoring of environmental xenobiotics in fish. Environ Health Perspect 71:105-119

Liu T-Y, Cheng SL, Ueng T-H, Ueng Y-F, Chi C-W (1991) Comparative analysis of aromatic DNA adducts in fish from polluted and unpolluted areas by the ^{32}P-postlabeling analysis. Bull Environ Contam Toxicol 47:783-789

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature (London) 227:680-685

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein determination with Folin phenol reagent. J Biol Chem 193:265-275

Nebert DW, Gelboin HV (1968) Substrate-inducible microsomal aryl hydrocarbon hydroxylase in mammalian cell culture. I. Assay and properties of induced enzyme. J Biol Chem 243:6242-6249

Omura T, Sato R (1964) The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein. J Biol Chem 239:2370-2378

Payne JF, Fancey, LL, Rahimtula AD, Porter EL (1987) Review and perspective on the use of mixed-function oxygenase enzymes in biological monitoring. Comp Biochem Physiol 86C:233-245

Park SS, Miller H, Klotz AV, Kloepper-Sams PJ, Stegeman JJ, Gelboin HV (1986) Monoclonal antibodies to liver microsomal cytochrome P-450E of the marine fish *Stenotomus chrysops* (scup): cross reactivity with 3-methylcholanthrene induced rat cytochrome P-450. Arch Biochem Biophys 249:339-350

Pohl R, Fouts JR (1980) A rapid method for assaying the metabolism of 7-ethoxyresorufin by microsomal subcellular fractions. Anal Biochem 107:150-155

Schoor WP, Williams DE, Takahashi N (1991) The induction of cytochrome P-450-IA1 in juvenile fish by creosote-contaminated sediment. Arch Environ Contam Toxicol 20:497-504

Stegeman JJ (1989) Cytochrome P450 forms in fish: catalytic, immunological and sequence similarities. Xenobiotica 19:1093-1110

Stegeman JJ, Lech JJ (1991) Cytochrome P-450 monooxygenase systems in aquatic species: Carcinogen metabolism and biomarkers for carcinogen and pollutant exposure. Environ Health Perspect 90:101-109

Towbin H, Staehelin T, Gordon J (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 76:4350-4353

Ueng T-H, Ueng Y-F, Park SS (1992) Comparative induction of cytochrome P-450-dependent monooxygenases in the livers and gills of tilapia and carp. Aquat Toxicol 23:49-64