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Characterization of hepatic flavin monooxygenase from the marine teleost turbot (*Scophthalmus maximus* L.)

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1. The presence and properties of flavin monooxygenase (FMO) in liver of the marine teleost, turbot (*Scophthalmus maximus*) were examined in relation to organic xenobiotic metabolism and osmoregulation.

2. Hepatic microsomes of sexually mature fish contained NADPH-dependent FMO as evidenced by the conversion of *N*,*N*-dimethylaniline (DMA) to DMA-*N*-oxide, and immunorecognition of single bands (approximate apparent molecular weight of 55 kDa) by antibodies to mammalian FMO 1 and FMO 2. Additionally, Northern analysis using a full-length cDNA probe to mammalian FMO 1 revealed a single hybridizing band of approximately 2·5 kb.

3. No significant differences were seen between male and female turbot FMO with respect to DMA *N*-oxidase activity, levels of immunoreactive protein (with anti-FMO 1 or anti-FMO 2) and gene expression (hybridizing mRNA).

4. Hepatic microsomal DMA *N*-oxidase activity was inhibited by methimazole (an FMO substrate) and trimethylamine (TMA), but not by piperonyl butoxide (a P450 inhibitor). Inhibition by TMA is indicative of a role for FMO in osmoregulation, catalysing the conversion of TMA to TMA *N*-oxide. DMA *N*-oxidase activity was optimal at pH 8·8 and 25°C, and displayed Michaelis–Menten kinetics with respect to DMA (apparent $Km = 88\ \mu M$).

Introduction

Hepatic monooxygenation, catalysed by cytochrome P450 or flavin monooxygenase (FMO), is an important biotransformation pathway for the metabolism of many organic xenobiotics, including pollutants. Whereas P450 has been studied extensively in fish and other aquatic organisms (Livingstone 1991, Stegeman and Hahn 1994), much less is known of the exogeneous or endogenous functions of FMO (Schlenk 1993). Like mammals, FMO in fish can both detoxify xenobiotics and activate certain compounds to more toxic products, e.g. conversion of aldicarb to aldicarb sulphoxide by hepatic microsomes of rainbow trout (*Oncorhynchus mykiss*) (Schlenk and Buhler 1991a). FMO requires NADPH and O_2 for catalysis, and exists in a stable activated, high-energy state independent of substrate binding (Ziegler 1993). The 4-α-hydroperoxy side-chain on the flavin molecule of FMO (formed by the addition of electrons and O_2) is susceptible to attack by weak nucleophiles such as various tertiary amines and thioethers, so defining its substrate specificity (Ziegler 1988).Thus, a characteristic catalytic property of FMO in both mammals and fish

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is oxidation of the xenobiotic *N*,*N*-dimethylaniline (DMA) to the *N*-oxide (Ziegler and Pettit 1964, Schlenk 1993).

Little is known of the endogenous functions of FMO in fish, but an osmoregulatory role has been indicated in salinity adaptation (Pang *et al.* 1977). Specifically, FMO is proposed to catalyse the metabolism of trimethylamine (TMA) to the osmolyte TMA *N*-oxide (Schlenk 1993). The latter *N*-oxide accounts for some 1–3% of the body dry weight of marine teleost fish, but is very low in freshwater fish (Strøm 1980). Induction of the enzyme and an osmoregulatory role, are indicated from TMA and salinity exposure studies. Thus, TMA *N*-oxidase activity in liver of eel (*Anguilla japonica*) and guppy (*Poecilia reticulata*) was markedly increased with transfer of the fish from freshwater to seawater (Daikoku *et al.* 1988). Further elevation of the TMA *N*-oxidfase activity occurred on subsequent exposure to TMA by injection or through the diet.

FMO exists in multiple forms in mammals. To date, five isoforms have been identified by their amino acid sequences (termed FMO 1, FMO 2, etc., Hines *et al.* 1994), and are distributed in organs such as liver, kidney and lung. The occurrence of some of these isoforms has been indicated in marine fish (Schlenk 1993), e.g. antibodies to mammalian FMO 1 and FMO 2 recognized proteins of similar molecular weight in microsomes of various tissues, including liver, of *O. mykiss* (Schlenk 1989). Similarly, using mammalian cDNA probes, the expression of both FMO 1 and FMO 2 mRNA was indicated in liver of dogfish shark (*Squalas acanthus*) and striped bass (*Morone saxatilis*) (Schlenk 1993).

The aim was to characterize FMO in liver of the marine teleost turbot (*Scophthalamus maximus*), a species available both farm reared (larvae through to mature adults) and from the wild, which offers potential for xenobiotic exposure and osmoregulatory studies. FMO was characterized from male and female fish in terms of gene expression, multiple forms (using a number of mammalian cDNA and antibody probes) and catalytic properties.

Materials and methods

Animals

Sexually mature (length 24·5–47·0 cm) feral *S. maximus*, caught in local waters off Plymouth, UK, were maintained in large tanks with continuous flowing seawater at 12–15°C for up to 3 months before biochemical analysis. They were fed on a selection of freshly killed local fish, but were starved for 3 days prior to killing. Fish were killed instantaneously by a blow to the head, and the livers immediately dissected and used.

Chemicals

DMA was obtained from Aldrich Chemical Co. (Milwaukee, USA) and recrystallized by the method of Schlenk and Buhler (1989). All biochemical and most molecular biology reagents, including β-nicotinamide adenine dinucleotide phosphate reduced form (NADPH), phenylmethylsulphonyl-fluoride (PMSF), sodium dodecylsulphate (SDS), molecular weight markers for SDS-polyacrylamide gel electrophoresis (PAGE), methimazole, piperonyl butoxide, diethyl pyrocarbonate (for RNA extraction and Northern blotting), and anti-sheep and anti-goat IgG alkaline phosphatase conjugates were from Sigma Chemical Co. (Poole, UK). Other chemicals, including ethylenediaminetetraacetic acid (EDTA), were of AnalaR grade, or equivalent, and obtained from Merck (Harlow, UK). Nitrocellulose paper was from Amersham (Little Chalfont, UK), and RNAzol® from Biogenesis (Poole, UK). [α-^{32}P]-deoxycytosine triphosphate (dCTP) was obtained from NEN-Dupont (Boston, USA).

Microsomal preparation for enzyme studies

All sample procedures were carried out at 4°C. Dissected liver was homogenized in a 1:4 wet weight: buffer volume ratio of 100 mM Tris–HCl, pH 7·6, containing 0·15 M KCl, 1 mM EDTA, 0·1 mM PMSF (added in minimal volume of ethanol), using an electrically driven Potter–Elvehjem homogenizer. The homogenate was centrifuged at 20 000 g, for 20 min, and the resulting supernatant spun at 100 000 g

for 90 min. The microsomal (100 000 g) pellet was resuspended in 100 mM K_2HPO_4/KH_2PO_4, pH 8·0, containing 20% w/v glycerol and 1 mM EDTA. This was used as the source of enzyme activity immediately, or stored at − 70°C prior to Western blotting analysis. Microsomes from individual livers were used for the sexual comparison studies, whereas pooled microsomes from mixed sexes were used for the enzyme kinetic and inhibitor studies (see below).

Enzyme activity studies

DMA *N*-oxidase activity was assayed by the method of Schlenk and Buhler (1991b), which was a modification of the original method of Ziegler and Pettit (1964). Contained in a final reaction volume of 1 ml were 50 mM Tris–HCl, pH 8·4, 0·5 mM NADPH, 2 mM DMA and 1 mg microsomal protein. The reaction was initiated by the addition of DMA, and the reaction mixture incubated at 20°C for 30 min. DMA *N*-oxide was indirectly quantified at 420 nm using an extinction coefficient of $8{\cdot}2\,mM^{-1}\,cm^{-1}$) using a Varian Cary 1 spectrophotometer. Enzyme assays were carried out in duplicate, and experiments were performed varying DMA concentration, temperature and pH (see text). The apparent Michaelis constant (K_m) for DMA was calculated by Lineweaver–Burk plot using a weighted, least-squares regression (Enzpack 3, Biosoft, UK). Additionally, the effects of competing FMO substrates (methimazole, TMA) and an inhibitor of P450 (piperonyl butoxide) were examined. Methimazole, TMA and piperonyl butoxide were directly added prior to initiation of the reaction by DMA (5 min pre-incubation). Incubations contained inhibitor concentrations of 2 mM. Protein was measured by the method of Lowry *et al.* (1951).

Western blot analysis

SDS-PAGE was carried out according to the method of Laemmli (1970). Microsomal preparations were diluted with distilled water to a protein concentration of 5·7 mg/ml and boiled for 2–3 min with an equal volume of 0·125 M Tris–HCl, pH 6·8, containing 4% SDS (w/v), 20% glycerol (v/v), 10% 2-mercaptoethanol (v/v) and 0·001% bromophenol blue (w/v). Aliquots (43 μg protein) of the mixture were then loaded onto a 10% SDS polyacrylamide gel and run using an Atto mini-gel system (Genetic Research Instrumentation, Dunmow, UK) for 40 min, followed by semi-dry transfer onto nitrocellulose paper (Towbin *et al.* 1979). Immunoreactive microsomal proteins were visualized using polyclonal sheep anti-pig liver FMO 1 IgG fraction (1 : 1000 v/v) and polyclonal goat anti-rabbit lung FMO 2 IgG fraction (1 : 1000 v/v) with subsequent incubation with anti-sheep or anti-goat IgG (whole molecule) alkaline phosphatase conjugate (1 : 3000 v/v). Purification and subsequent raising of antibodies to each of the purified proteins were performed using previously published methods (Ziegler and Mitchell 1972, Williams *et al.* 1985). Purified proteins (FMO 1 and FMO 2) were used as positive controls for immunoblots. Molecular weights of *S. maximus* immunoreactive proteins were determined from molecular weight markers transferred to the membrane. Protein bands were semiquantified using a Kontron image analyser (Image Processing System, Watford, UK).

Northern blot analysis

Total RNA was extracted from individual fresh livers by a single-step method of Chomczynski and Sacchi (1987) using guanidium thiocyanate/phenol/chloroform extraction (RNAzol®) according to the manufacturers (Biogenesis Ltd.) instructions, and electrophoretically fractionated on a 1·5% agarose gel. RNA was then transferred to nitrocellulose under vacuum (VacuGene pump, Pharmacia-LKB, St. Albans, UK) and hybridized overnight at 47°C with a full-length cDNA probe encoding FMO 1, radiolabelled by random primer extension to a specific activity of $>10^8$ cpm/μg with [α-^{32}P]-dCTP (Feinberg and Vogel 1983, Feinberg and Vogel 1984). The FMO 1 cDNA probe was synthesized using polymerase chain reaction amplification. Both sense and anti-sense primers based on the sequence reported by Lawton *et al.* (1990) were used to amplify the FMO 1 cDNA from a total cDNA library made from 1·0 μg total liver rabbit liver RNA. The resulting FMO 1 cDNA fragment spanning from nucleotides 34 to 1674 was cloned into the SMAI site of pUC19 and the identity of the FMO cDNA verified by complete DNA sequence analysis.

After incubation with the FMO 1 cDNA probe the membrane was washed three times at 47°C with 0·5 × SSC containing 0·1% SDS and exposed to Kodak XAR film for 2 weeks. Bands were semi-quantitated using BioRad GS670 imaging densitometer (Biorad Inc., New York, USA). Each sample was normalized by dividing the area of the 18S RNA band visualized by ethidium bromide staining on the membrane prior to hybridization.

Statistics

Values are presented as mean ± SEM. Differences between groups of values were compared using a student's *t*-test, and effects of various inhibitors were compared using Dunnet's multiple range test, which compares multiple samples to a single control. $p < 0{\cdot}05$ was accepted as statistically significant. The correlation matrix was determined using StatView 512 + ®.

Results

Microsomal DMA *N*-oxide activity, microsomal immunoreactive protein and whole-tissue hybridizing RNA were detected in the liver of both male and female *S. maximus* (table 1). No statistically significant differences were observed for any of these measurements between male and female fish. Polyclonal antibodies raised against mammalian FMO 1 and FMO 2 recognized single proteins of approximately 55 kDa apparent molecular weight (figure 1). The level of recognition, or protein amounts, were indicated to be greater for anti-FMO 2 than anti-FMO 1, but the differences were not statistically significant (data not shown). Single bands of similar apparent molecular weight were also seen for the two positive controls, pig liver FMO 1 (figure 1 A, lane 8) and rabbit lung FMO 2 (figure 1 B, lane 1).Hybridization of total hepatic RNA with a full-length cDNA for FMO 1 revealed a single band of approximately 2·5 kb (figure 2). Expression of the 55 kDa protein recognized by anti-FMO 1 did not correlate with that of the 55 kDa protein recognized by anti-FMO 2 individual males (table 2). However, expression of the 55 kDa protein recognized by anti-FMO 1 did correlate with FMO activity and the 2·5 kb transcript hybridizing to FMO 1 cDNA. Conversely, expression of the 55 kDa protein recognized by anti-FMO 2 did not correlate with FMO activity or the 2·5 kb transcript hybridizing to FMO 1 cDNA. In females, expression of proteins recognized by both FMO 1 and FMO 2 positively correlated with each other as well as FMO activity and the 2·5 kb mRNA transcript (table 2).

DMA *N*-oxidase activity characterization studies were carried out on the pooled hepatic microsomes of male and female *S. maximus*. The activity showed Michaelis–Menten kinetics with respect to dependence on DMA concentration, with a correlation of 0·97 observed for the Lineweaver–Burk plot (figure 3). The apparent K_m for DMA was 88 μM. Somewhat sharp profiles were observed for the dependence of DMA *N*-oxidase on both temperature (figure 4) and pH (figure 5) with optima of respectively 25°C (sharp decline at 37°C) and pH 8·8 (range 8·5–9·2). DMA *N*-oxidase activity was inhibited by trimethylamine and methimazole, 43 and 75% respectively, but stimulated 45% by piperonyl butoxide (table 3).

Discussion

FMO catalyses the monooxygenation of tertiary amines and a variety of other heteroatom-containing 'soft' nucleophilic compounds. In fish tissues, such as liver, it is indicated to have a role in both exogenous and endogenous metabolism respectively, organic xenobiotic metabolism (e.g. *N*-oxidation of DMA), and osmoregulation (*N*-oxidation of TMA) (see Introduction).

The present study was carried out to improve the understanding of FMO presence and function in a marine fish species, the turbot *S. maximus*. The liver was chosen as a major organ of organic xenobiotic metabolism in fish (Varanasi *et al.* 1989, Walker and Livingstone 1992, Schlenk 1993) and one in which FMO activity is affected by salinity (Charest *et al.* 1988, Daikoku *et al.* 1988). The presence and expression of a functional FMO gene/enzyme in hepatic microsomes of *S. maximus* was shown by the detection of hybridizing mRNA species, immunoreactive protein, and enzyme activity with DMA as substrate. The specific activity of hepatic FMO in *S. maximus* (0·2–0·3 nmol min^{-1} mg^{-1} microsomal protein) was lower than that of other fish (~ 0·740 for *O. mykiss* (Schlenk and Buhler 1991b), 1·2 for the smooth dogfish (*Squalus acanthus*) (Schlenk and Li-Schlenk 1995), and 0·05–1·15 for four species of elasmobranch (Goldstein and Dewitt-Harley 1973)). Catalytic activity

Table 1. Levels of *N*,*N*-dimethylaniline (DMA) *N*-oxidase activity and anti-FMO 1 and anti-FMO 2 immunoreactive protein in hepatic microsomes, and FMO 1 mRNA in whole liver of male and female *Scophthalamus maximus*.

Measurement	Male	Female
DMA *N*-oxidase activity (pmol/min/mg protein)	254·0 ± 65·4	280·0 ± 142·9
FMO 1 protein†	23·7 ± 6·3	17·9 ± 5·2
FMO 2 protein†	30·8 ± 4·2	26·4 ± 5·1
FMO 1 mRNA†	67 ± 10	80 ± 15

Values are mean ± SEM ($n = 3$); no differences observed in any measurement between male and female ($p > 0{\cdot}05$).

† Arbitrary units (see Materials and methods for details of probes).

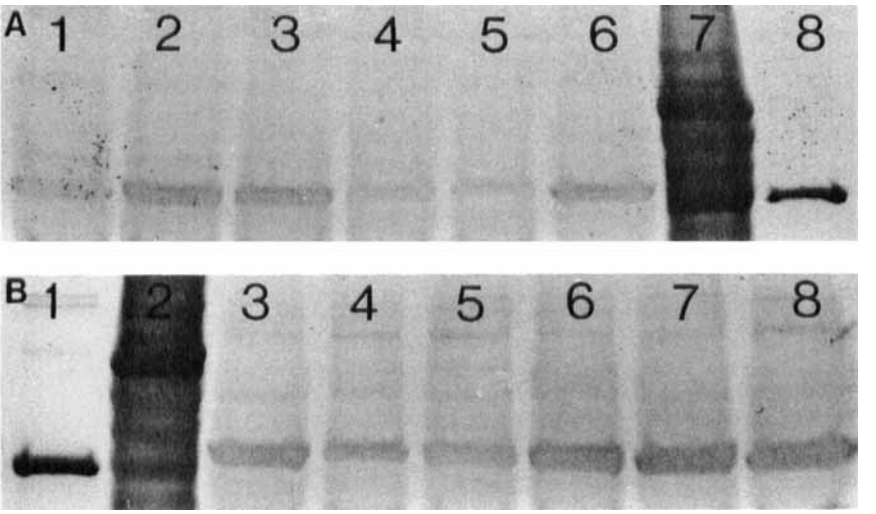

Figure 1. Western blot analysis of hepatic microsomes from individual male and female *Scophthalmus maximus* using polyclonal antibodies to mammalian FMO 1 (A: lanes 1–3, male; 4–6, female; 7, mol. wt. markers; and 8, pure pig FMO 1) and FMO 2 (B; lanes 1, pure rabbit FMO 2; 2, mol. wt. markers; 3–5, female; and 6–8, male); 43 μg protein was loaded per lane.

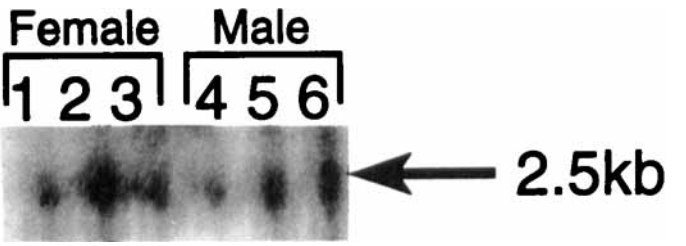

Figure 2. Northern blot analysis of total hepatic RNA from individual male and female *Scophthalmus maximus* probed with FMO 1 cDNA: lanes 1–3, hepatic RNA from female; and 4–6, hepatic RNA from male; 10 μg RNA was loaded per lane. Molecular weight was determined using ethidium bromide-stained RNA markers (Promega) 0·36–9·49 kb.

Table 2. Correlation coefficients for the intercomparison of hepatic microsomal anti-FMO 1 and anti-FMO 2 immunoreactive protein amount, *N*,*N*-dimethylanaline *N*-oxidase activity and mRNA hybridizing to FMO 1 cDNA in male and female *Scophthalmus maximus*.

Sex	FMO 1	FMO 2	Enzyme activity
Male FMO 1	×		
Male FMO 2	−0·09	×	
Male enzyme activity	1·00	−0·08	×
Male FMO 1 mRNA	0·99	−0·14	0·99
Female FMO 1	×		
Female FMO 2	0·99	×	
Female enzyme activity	0·99	0·99	×
Female FMO 1 mRNA	0·99	0·99	1·00

Data from table 1.
× Invalid comparison.

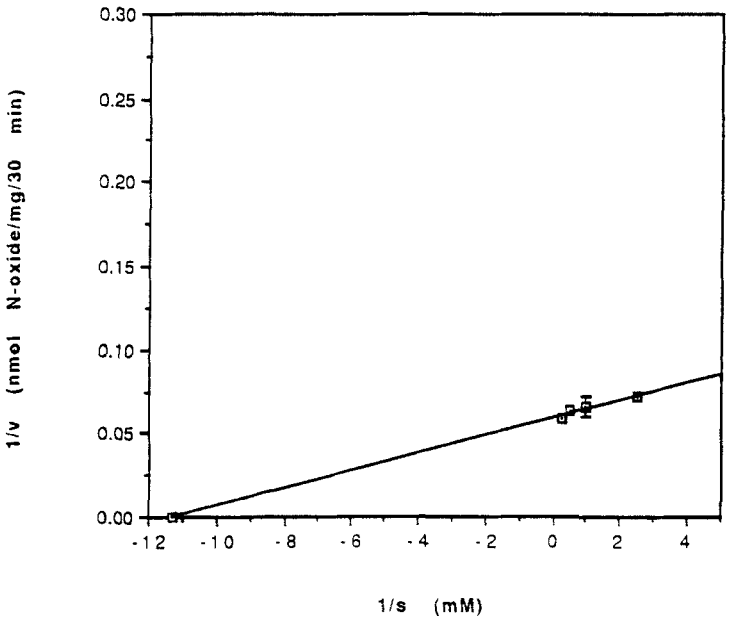

Figure 3. Lineweaver–Burke plot of the dependence of *N*,*N*-dimethylaniline *N*-oxidase activity on *N*,*N*-dimethylaniline substrate concentration in hepatic microsomes of *Scophthalmus maximus*. Each value is the mean of three assays (± SEM; not shown if less than symbol size). Other reaction conditions: 0·5 mM NADPH, 50 mM Tris–HCl, pH 8·4, 20°C. Activities were determined from pooled samples from male and female.

towards TMA, and a possible role in osmoregulation, is indicated from the inhibition of microsomal DMA *N*-oxidase activity by TMA. Hepatic microsomal TMA *N*-oxidase activity has been measured directly in a number of teleost fish species, including *A. japonica* and *P. reticulata* (Daikoku *et al.* 1988) and cod (*Gadus morhua*) (Ágústsson and Strøm 1981).

No marked differences in DMA *N*-oxidase activity were seen between male and female *S. maximus*, consistent with most previous fish studies (Schlenk 1993), but differing from mammalian liver, which had higher FMO levels in the male than female rat (Dannan *et al.* 1986) and the reverse in mouse (Duffel *et al.* 1981).

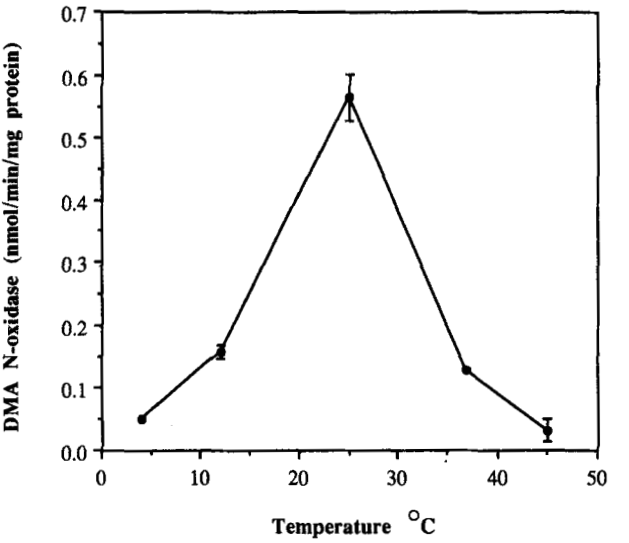

Figure 4. Dependence of *N,N*-dimethylaniline *N*-oxidase activity of hepatic microsomes of *Scophthalmus maximus* on incubation temperature. Each value is the mean of two assays (± range; not shown if less than symbol size). Other assay conditions: 2 mM *N,N*-dimethylaniline, 0·5 mM NADPH, 50 mM Tris-HCl, pH 8·4.

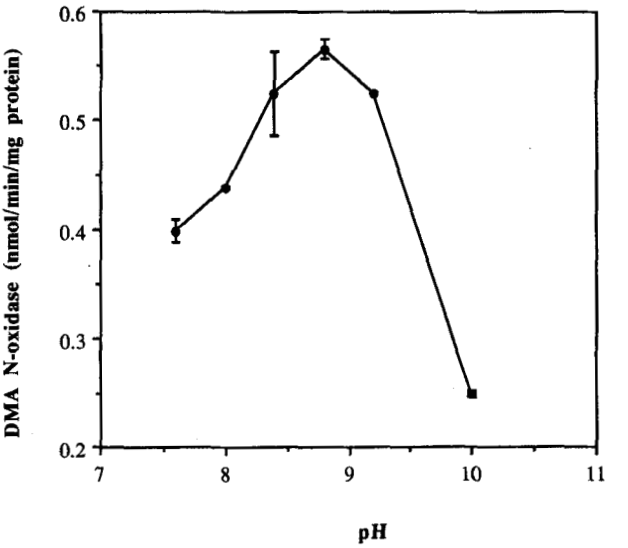

Figure 5. Dependence of *N,N*-dimethylaniline *N*-oxidase activity of hepatic microsomes of *Scophthalmus maximus* on pH (50 mM Tris–HCl). Each value is the mean of two assays (± range; not shown if less than symbol size). Other assay conditions: 2 mM *N,N*-dimethylaniline, 0·5 mM NADPH, 20°C.

Table 3. Effects of competitive FMO substrates (methimazole, trimethylamine) and inhibitor of P450 (piperonyl butoxide) on *N*,*N*-dimethylaniline *N*-oxidase activity of hepatic microsomes of *Scophthalamus maximus*.

Control	Methimazole	Trimethylamine	Piperonyl butoxide
100 ± 11	25 ± 5†	57 ± 12†	145 ± 21†

Activities expressed as % of the control DMA *N*-oxidase activity (240 ± 26 pmol/min/mg protein), values are mean ± SEM ($n = 3$).

†$p < 0{\cdot}05$ compared with control.

No differences in FMO protein levels with sex were seen in *S. maximus*. However, the correlation of protein recognized by anti-FMO 1 and anti-FMO 2 in the female, and the absence of the same correlation in the male, indicates that sexual differences may be present. In studies with *O. mykiss*, hepatic isoform differences of FMO with sex have been indicated (Schlenk and Buhler 1993).

The physico-chemical and enzyme kinetic properties of the *S. maximus* hepatic microsomal FMO are similar to those observed for other fish and mammalian species. The relatively sharp pH profile for DMA *N*-oxidase activity of 8·5–9·2 (optimum pH 8·8) compares well with that of 8·4–9·2 for *O. mykiss* (Schlenk and Buhler 1991b) and a pH optimum of 8·4 for the porcine enzyme (Ziegler 1988). Similar high pH optima of 8·2 and 9·0 were seen for TMA *N*-oxidase activity for respectively *G. morhua* (Ágústsson and Strøm 1981) and the nurse shark (*Ginglymostoma cirratum*) (Goldstein and Dewitt-Harley 1973). The marked temperature sensitivity of DMA *N*-oxidase activity of *S. maximus* (optimum of 25°C decreasing by 77% at 37°C) is very similar to that found for *O. mykiss* (optimum of 25°C decreasing by 87% at 35°C (Schlenk and Buhler 1991b). High sensitivity to heat inactivation is a characteristic of mammalian FMO 1 (Ziegler 1988), possibly indicating a structural homology of this isoform with fish FMO(s). Hepatic microsomes of *S. maximus* showed Michaelis–Menten kinetics with respect to DMA *N*-oxidation, consistent with results for *O. mykiss* (Schlenk and Buhler 1991b), but with a much lower apparent K_m for DMA, *viz.* at the same pH (8·4) and similar NADPH concentration (88 μM) (*S. maximus*) compared with 1·2 mM (*O. mykiss*). However, the apparent K_m for hepatic FMO activity in the elasmobranch *S. acanthus* (44 μM) (Schlenk and Li-Schlenk 1995) was two-fold lower than *S. maximus*. The marked intra- and interspecies variation in both specific activities and /or apparent K_m's for FMO in fish is striking and may indicate an enzyme that is regulated in relation to both endogenous (TMA) and exogenous factors (salinity, temperature, etc.). For example, hepatic TMA *N*-oxidase activity was enhanced seven and 224 times in *A. japonica* exposed to TMA and increased salinity respectively (Daikoku *et al.* 1988).

DMA can be metabolized by both P450-dependent (demethylation) and FMO-dependent (*N*-oxidation) pathways in mammalian hepatic microsomes (Houdi and Damani 1985). The absence of inhibition of *S. maximus* DMA *N*-oxidase activity by the P450 inhibitor piperonyl butoxide is therefore consistent with other vertebrate studies, including fish (Schlenk *et al.* 1991b, Schlenk 1993), and indicates that the reaction is not catalysed by P450. The fact that piperonyl butoxide stimulated *S. maximus* DMA *N*-oxidase activity is most likely due to inhibition of the competing P450-dependent pathway, so directing DMA towards increased FMO-dependent metabolism. In contrast with this result, other P450 inhibitors

(SKF-525A, carbon monoxide, *N*-benzylimidazole) did not stimulate FMO activity in other fish species (Goldstein and Dewitt-Harley 1973, Schlenk and Buhler 1991b). The inhibition of *S. maximus* DMA *N*-oxidase activity by two substrates of FMO (methimazole and TMA) indicate that the reaction is FMO-catalysed. Studies on DMA *N*-oxidase activity in *O. mykiss* (Schlenk and Buhler 1991b) and *S. acanthus* (Schlenk and Li-Schlenk 1995) have demonstrated that the inhibition by methimazole is non-competitive, whereas that of TMA is competitive. It is unclear how TMA and methimazole inhibit hepatic FMO activity in *S. maximus*, but the occurrence of competitive inhibition by TMA and non-competitive inhibition by methimazole in several other fish species suggests structural similarities in the active sites for fish FMOs.

In addition to functional similarities, recognition of *S. maximus* microsomal proteins by antibodies raised against FMO 1 and FMO 2 (Hines *et al.* 1994). Indicate structural homology between the *S. maximus* and mammalian FMOs. Each antibody recognized a single band of protein with similar apparent molecular weight (55 kDa for *S. maximus*) as the purified pig liver or rabbit lung enzymes. Although temperature sensitivity studies indicated a functional homology of the *S. maximus* enzyme to FMO 1, antibodies to FMO 2 were indicated to have a slightly higher degree of recognition than antibodies to FMO 1. In *O. mykiss* and *S. acanthus*, antibodies to FMO 1 and 2 recognized hepatic proteins of the same apparent molecular weight with equal intensity (Schlenk 1993), where as in rabbit, FMO 1 and 2 are immunochemically distinct (Ziegler 1988).

The order of FMO isozyme evolution is not known, but the occurrence of FMO 1 is tissues of all mammalian species examined to date suggests that this form may be one of the early mammalian forms of FMO. Thus, a full-length cDNA coding for FMO 1 was used to probe mRNA isolated from *S. maximus* liver. The presence of a single band of approximately 2·5 kb, concomitant with the presence of FMO protein and activity in individual animals, indicates that the identity of the hybridizing band is FMO mRNA. The Western and Northern analyses both gave single bands, possibly indicating the presence of a single gene and enzyme. However, in the former case recognition by both FMO 1 and FMO 2 antibodies could indicate either a single isoenzyme with both epitopes, or two isoenzymes of very similar molecular weight. The lack of correlation between isoforms recognized by anti-FMO 1 and anti-FMO 2 in the males suggests that isoform differences may, in fact, be present in male *S. maximus*. In the case of hepatic microsomes of *O. mykiss*, using the same mammalian antibodies, two bands of different apparent molecular weight (61 and 57 kDa) were detected (Schlenk and Buhler 1993).

In summary, molecular biological and enzymological studies have shown the presence of FMO in hepatic microsomes of the marine teleost *S. maximus*. Inhibition of DMA *N*-oxidase activity by methimazole and TMA indicate a wide substrate specificity for the enzyme, consistent with known FMO properties and indicative of roles in biotransformation and osmoregulation. Recognition of the *S. maximus* FMO by mammalian cDNA and protein probes suggests structural and genetic homology between the piscine and mammalian enzymes.

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