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Cytochrome P-450 Isozymes and Monooxygenase Activity in Aquatic Animals

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The roles of different forms of cytochrome P-450 in activation and deactivation of toxic chemicals, synthesis and breakdown of steroid hormones, and other functions, indicate the significance of these enzymes. Monooxygenase systems have been studied in species from several phyla of aquatic organisms. However, cytochrome P-450, the dominant catalyst in xenobiotic monooxygenase activity, is best studied in fish. Forms of cytochrome P-450 have been purified from several teleost species, including scup (*Stenotomus chrysops*), rainbow trout (*Salmo gairdneri*), and cod (*Gadus morhua*). Cytochrome P-450E from scup, cytochrome P-450 LM_{4b} from trout, and cytochrome P-450c from cod have properties similar to each other and appear to be homologous hydrocarbon or BNF-inducible isozymes. Partially purified cytochrome DBA-P-450-I from little skate, *Raja erinacea*, is possibly an elasmobranch counterpart of these teleost forms. Cytochrome P-450E from scup is immunochemically related to the major BNF-inducible isozyme (cytochrome P-450c or BNF-B) in rats, indicating homology between the fish and mammalian BNF-inducible isozymes. Several other cytochrome P-450 forms with interesting or unusual properties have been purified from aquatic species. Mammalian homologs are not yet known for these isozymes. Further studies of cytochrome P-450 forms in aquatic species should establish additional homologies and the regulation of these forms by chemical and biological variables, possibly providing fundamental insights into the function and evolution of these proteins.

Comparative Biochemical Toxicology

Diverse fields, including environmental toxicology, pharmacology and therapeutics, and chemical carcinogenesis, are unified in the effort to understand and influence chemical-biological interactions. Biotransformation is a central feature of such interactions, determining the biological activity of many foreign organic compounds. Since R. T. Williams' seminal work on foreign compound disposition (*1*), an enormous literature has accrued describing pathways of foreign compound metabolism, and a strong link between the metabolism and biological activity of many foreign compounds has been established. Drugs and chemotherapeutic agents (*2*), man-made pollutants such as ubiquitous polychlorinated biphenyls (*3*), natural products such as 4-ipomeanol (*4*), and naturally occurring or anthropogenic chemical carcinogens (*5*) are either activated or inactivated by the process of biotransformation.

Oxidative metabolism of xenobiotics is often the first step in biotransformation. Numerous reviews and books over the past 20 years discuss the plethora of mammalian catalysts and mechanisms that function in the oxidation of organic xenobiotics (*6,7*). Comparatively little is known concerning the mechanisms of oxidative xenobiotic metabolism in nonmammalian species. A point made nearly 20 years ago by Williams (*8*)—that definite conclusions about relationships among chemicals, species, and metabolic routes were not possible—is to a large extent still true. However, knowledge concerning the catalysts of oxidative metabolism and relationships between these in various species is increasing. In both mammalian and some nonmammalian groups, the dominant catalysts for oxidative transformation of xenobiotics are comprised of the cytochrome P-450 family of enzymes. While the occurrence of cytochrome P-450 isozymes and their functions in some species is well established, in many nonmammalian species formal demonstration of a role for these catalysts in xenobiotic effects is lacking.

Among important reasons for pursuing comparative studies of xenobiotic metabolism are to determine the capacity of individuals, populations, or species to metabolize foreign compounds, and their susceptibility to effects of compounds that are deliberately or inadvertently applied; to identify the mechanisms and regulation of the catalysts; and to establish the phylogenetic continuity and probable evolution of the catalysts and their regulatory systems. If our objectives are to understand the toxicology of aquatic pollutants, then the mechanisms by which toxicants are converted to more or less active derivatives, and the regulation of those processes in aquatic species, must be understood. The same need underlies the use of aquatic vertebrates or inverte-

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brates as models for toxicological or carcinogenesis research. A major objective of research on model preparations is to understand the significance of chemical-biological interactions in humans. In this regard studies on rats, mice, and rabbits also fall in the realm of comparative toxicology. Defining the processes and catalysts in lower vertebrates and invertebrates will help establish the common features of mechanisms involved in chemical-biological interactions. The objective of this brief review is to consider recent progress in studies of the dominant monooxygenase catalysts, the cytochromes P-450, in aquatic species.

Monooxygenase Function in Microsomes

The basic characteristics of microsomal systems and their function in aquatic species have been treated in detail in several reviews (*9–11*). Cytochrome P-450 functions in association with microsomal proteins that transfer electrons to reduce cytochrome P-450 during its catalytic cycle. These components include NADPH-cytochrome P-450 reductase, cytochrome b_5 (which also functions in fatty acid desaturation), and NADH-cytochrome b_5 reductase. The levels of cytochrome P-450 and the electron transfer components in representative aquatic species from several phyla, shown in Table 1, indicate the ranges often seen in these values. In general, the levels of these various components in nonmammalian vertebrates approach and in some cases exceed those commonly seen in mammals, while the levels in invertebrates are lower.

Several well-known points deserve reiteration here. Microsomal content of cytochrome P-450 determined spectrally reflects the sum of all forms present and does not reveal the isozyme composition nor any feature pertaining to a specific isozyme. Second, the spectrally determined cytochrome P-450 content does not indicate catalytic function, as demonstrated by comparing levels of AHH activity and cytochrome P-450 content (Table 1). Microsomal activity will depend on the relative abundance of different isozymes and their functions in the membrane. Degraded (inactive) cytochrome P-450 can also be detected spectrally, and its presence should be considered in evaluating microsomal function. Third, the level of NADPH-cytochrome P-450 reductase does not necessarily indicate the efficiency of required electron transfer to cytochrome P-450 by this protein. However, in some systems such as the spiny lobster, low levels of microsomal reductase activity are associated with inefficient monooxygenase function *in vitro*. Spiny lobster reductase is inactivated during membrane preparation, and consequently NADPH will not support AHH activity (*14*). Yet cumene hydroperoxide does support a high AHH activity, showing that the cytochrome P-450 present is functional. The levels of cytochrome b_5 and cytochrome b_5 reductase also do not indicate the involvement of these components in providing a second electron to cytochrome P-450. Yet cytochrome b_5 in particular has been found to influence strongly some cytochrome P-450 functions *in vitro* in mammals (*15*) and recently, in fish (*16*). Similar functions could occur *in vivo* and complete evaluation of microsomal function thus requires that levels of these various functional components be measured.

The catalytic function of cytochrome P-450 in microsomal preparations has been evaluated in a large number of fish and invertebrate species. Most studies that have compared the function of microsomal systems in several species have employed a limited set of substrates, including those commonly used to characterize mammalian microsomal systems or purified isozymes (*17*). It must be emphasized that the activity in microsomal fractions seen with a given substrate does not necessarily indicate the function of this system with any other substrate. The activity with benzo(a)pyrene (AHH activity) shown in Table 1, for example, does not permit any inference about the metabolism of aminopyrine or testosterone, as these activities can be cata-

Table 1. Microsomal cytochrome P-450, electron transport components, and monooxygenase activity in aquatic species.

Microsome source[a]	Cytochrome P-450, nmole/mg	Cytochrome b_5, nmole/mg	NADPH-cyt-c (P-450) reductase, nmole/min/mg	NADH-cyt-c (b_5) reductase, nmole/min/mg	Aryl hydrocarbon hydroxylase, nmole/min/mg
Molluscs					
Mussel (dig.gl.)	0.10	0.04	8.1	41.6	0.011
Crustaceans					
Barnacle (dig.gl.)	0.11	0.02	29.9	112.0	0.043
Spiny lobster (dig.gl.)	0.91	—	4.3	—	ND[b]
Fish					
Scup (liver)	0.61	0.07	106.0	179.0	0.685
Rainbow trout (liver)	0.24	0.04	22.0	123.0	0.015
Mammal					
Rat (liver)	0.73	0.40	118	—	0.034

[a] Animals in each case were untreated. Data from authors' laboratory (*10, 12, 13*), except for spiny lobster (*14*). Data for rat are author's unpublished values. Dig.gl. is digestive gland. Species names in order are: *Mytilus edulis, Balanus eburneus, Panulirus argus, Stenotomus chrysops, Salmo gairdneri* and *Rattus rattus*.

[b] ND = not detectable.

lyzed principally by different forms of cytochrome P-450 (*18,19*). Furthermore, the metabolism of one substrate can change independently of another, if they are catalyzed by different forms of cytochrome P-450 that are differentially regulated. However, several activities can be catalyzed by the same form of cytochrome P-450, in which case activity with one substrate might reflect activity with additional substrates.

Monooxygenase Induction

Cytochrome P-450 systems are regulated by numerous environmental and biological variables (*6*). Patterns and mechanisms of induction by specific chemicals are better understood than is endogenous regulation. In mammals, the distinct patterns of induction caused by 3-methylcholanthrene (3-MC) and related compounds (*20,21*), by phenobarbital (PB) and related compounds (*22*), by pregnenolone-16α-carbonitrile (PCN), and by isosafrole (*24*) are well known.

Induction of microsomal activity in aquatic species by 3-MC and related compounds has been considered in detail in several reviews (*10,11*). This induction is very efficient in fish, resulting in increased levels of catalytic function with a suite of substrates, like the changes seen in mammals (Table 2). As discussed elsewhere in these proceedings, the induction by 3-MC-type inducers in fish involves *de novo* synthesis of cytochrome P-450 (*26*). The similarity of the responses suggests that similar isozymes of cytochrome P-450 are being regulated in teleost and mammalian species. Data presented below strongly support this idea.

In sharp contrast to 3-MC-type induction, induction by PB is either absent or greatly reduced in fish. A few studies have suggested that some response to PB-type compounds might occur (*17,27*), but as previously discussed (*10,11*), results of those studies are inconclusive. There is, in general, no indication that PB induction like that in mammals occurs in fish. The lack of a PB response could reflect the lack of structural genes for cytochrome P-450 isozymes related to the mammalian PB-induced isozymes, or it could reflect differences in regulatory processes for such genes. Establishing the nature of these differences could indicate the evolutionary basis for developing or maintaining a PB response.

Table 2. Induction of microsomal monooxygenase activity in vertebrate species.[a]

Microsome source	Aryl hydrocarbon hydroxylase, nmole/min/mg	Ethoxyresorufin *O*-deethylase, nmole/min/mg
Scup		
Control	0.250 ± 0.050	0.31 ± 0.09
3-MC	0.964 ± 0.070	4.69 ± 0.06
Rainbow trout		
Control	0.011 ± 0.0005	0.10 ± 0.07
3-MC	0.213 ± 0.005	3.63 ± 0.09

[a]Data from Stegeman et al. (*25*).

Cytochrome P-450 Isozymes in Mammals

Understanding the variability and regulation of specific catalytic functions in microsomes depends upon knowledge of the specific cytochrome P-450 forms involved. Establishing species similarities likewise requires a knowledge of the individual isozymes of cytochrome P-450. The occurrence of multiple cytochrome P-450 isozymes in mammalian species and their roles in specific catalytic functions have been reviewed extensively (*28,29*). The knowledge generated in the past 10 years on cytochrome P-450 in mammals has guided the investigation of these isozymes in aquatic systems. Comparison between cytochrome P-450 isozymes in different taxonomic groups necessarily includes reference to the abundant library of forms known in mammalian systems. As many as 14 isozymes of cytochrome P-450 have been identified in liver of rats, the species from which the most isozymes have thus far been purified and characterized. A listing of some of these isozymes, together with different designations and some characteristics, is provided in Table 3.

Establishing the distinctions or similarities between isozymes requires not only physicochemical characterization but also analysis of catalytic functions, immunological relatedness, and ultimately, primary sequence. Many of the isozymes indicated in Table 3 have multiple catalytic functions, but particular activities, e.g., AHH activity of cytochrome P-450c, are prominent with a given isozyme. Many of the forms that have been described in rat have counterparts that have been identified in rabbit and mouse. More recently, immunological relationships and primary sequences, determined directly or inferred from the nucleotide sequence of the mRNA or the genes for these proteins, have established the homology between some forms. As a key example, rabbit cytochrome P-450 form 6 and mouse cytochrome

Table 3. Cytochrome P-450 isozymes purified from rat liver.

Isozyme[a]	Other designations[b]	Fe^{2+}-CO λ_{max}, nm	M_r	Active inducer
P-450a	(UT-F; 3)	452	48,000	PB, 3-MC (low)
P-450b	(PB-B; PB4)	450	52,000	PB
P-450c	(BNF-B)	447	56,000	3-MC, BNF, PCB
P-450d	(ISF-G)	447	52,000	Isosafrole, 3-MC
P-450e	(PB-B; PB5)	450.6	52,500	PB
P-450f		447.5	51,000	
P-450g	(RLM 3)	447.5	50,000	
P-450h	(UT-A; 2c; RLM 5)	450.7	51,000	
P-450i	(UT-I; 2d; 15B)	449	50,500	
P-450j		451.5	51,500	Isoniazid
—	(PB-C; PB-1)	450	47,500	PB
—	(PCN-E; PB-2a)	450	51,000	PCN, PB

[a] Designations for isozymes P-450a through P-450j are according to Ryan et al. (*22, 24, 30–32*).

[b] Other designations are according to Guengerich et al. (*18*) and Waxman et al. (*28, 33*).

P_1-450 are counterparts of the BNF-inducible rat cytochrome P-450c (*34*).

Cytochrome P-450 Isozymes in Fish

Cytochrome P-450 isozymes from aquatic species are to date represented by far fewer forms than have been seen in mammals. Two fractions, DBA-P-450-I and DBA-P-450-II, were initially resolved from an elasmobranch, little skate (*Raja erinacea*) treated with dibenzanthracene (*35*). DBA-P-450-I had features like those prominent in microsomes of the dibenzanthracene-treated animals. More recently, there have been four or five fractions resolved from each of three teleost species, and several of these forms have been highly purified. In our studies we have resolved five distinct cytochrome P-450 fractions from the marine teleost scup, and three of these have been highly purified (*16,36*). In studies with rainbow trout there have also been five distinct fractions described, and these also are represented by three highly purified preparations (*37,38*). Four cytochrome P-450 fractions have been partially purified from the Atlantic cod (*39*). Designations for these forms or fractions and some of their characteristics are indicated in Table 4. In addition to the forms listed in Table 4, there have been two fractions resolved from rainbow trout by Arinc and Adali (*40*). These fractions have not yet been sufficiently characterized to permit comparisons with other forms. However, based on spectral characteristics, it is possible that fraction P-450-I of Arinc contains isozyme(s) equivalent to rainbow trout cytochrome(s) P-450 LM_4.

The physicochemical properties of the highly purified forms indicate that these are distinct isozymes in the various fish species. Reconstitution of catalytic function of several of the preparations from scup and trout supports this contention. The activities of highly purified isozymes and other fractions from these two species with several commonly used substrates are indicated in Table 5. The activities shown include three known to be induced in microsomes by 3-MC- or PAH-type inducers. Other activities have been estimated for one or another of these preparations but have not been analyzed for all of the isozymes represented and are not shown here.

Table 4. Hepatic cytochrome P-450 isozymes purified or partially purified from teleosts.

Species	Isozyme designation	Purity >10 nmole/mg	Fe^{2+}-CO λ_{max}	M_r
Scup[a]	P-450A	+	447.5	52,700
	P-450B	+	449.5	45,900
	P-450C	−	450	49–51,000[d]
	P-450D	−	451	50,000
	P-450E	+	447	54,300
Rainbow trout[b]	P-450 LM_1	−	449	50,000
	P-450 LM_2	+	449.5	54,000
	P-450 LM_3	−	447.5	56,500
	P-450 LM_{4a}	+	447	58,000
	P-450 LM_{4b}	+	447	58,000
Cod[c]	P-450a	−	447.5	55,000
	P-450b	−	447.5	54,000
	P-450c	+	448	58,000
	P-450d	+	448	56,000

[a] Data from Klotz et al. (*16,36*). Fractions are named in order of resolution from DEAE/Sephadex.

[b] Data from Williams and Buhler (38). Fractions are named in order of electrophoretic migration.

[c] Data from Goksøyr (*39*). Fractions are named in order of chromatographic resolution.

[d] Purity insufficient to accurately estimate M_r.

Roles in Bioactivation

Several of the isozymes in fish have been evaluated for formation of benzo(a)pyrene metabolites. Both scup cytochrome P-450E and trout cytochrome P-450 LM_{4b} form high percentages of benzo-ring dihydrodiols of BP when reconstituted with epoxide hydrolase (*36,38*). As seen in Table 6, dihydrodiols can comprise as much as 75% of the total metabolites formed by cytochrome P-450E. The efficient formation of the *trans*-7,8- dihydrodiol is particularly important, as an isomer of this product is the immediate precursor of the carcinogenic bay region 7,8-diol-9,10-epoxides (*41*). Thus, we propose that the function of cytochrome P-450E, cytochrome P-450 LM_{4b} and their counterparts would be responsible for initiation of carcinogenesis by some PAH in fish. This function would be consistent with the similar function of the related BNF-induced mammalian cytochrome P-450s (see below). Knowing the function and

Table 5. Reconstituted activities of teleost cytochrome P-450 isozymes.

	Activity, nmole/min/nmole P-450				
				Testosterone hydroxylase	
Isozyme or fraction	AHH	EROD	ECOD	Major product	Activity
Scup[a]					
P-450A	0.16	ND[c]	0.40	6β[e]	0.80
P-450B	0.001	d	0.05	15α	0.07
P-450C	0.001	ND	—	16α	—
P-450D	0.001	—	0.06	16α and 16β	0.15
P-450E	0.9	7.1	2.2	6β	0.04
Trout[b]					
P-450LM_1	0.01	0.02	0.05	—[f]	—
P-450LM_2	0.02	0.74	0.05	6β	0.011
P-450LM_3	0.01	0.02	0.05	—	—
P-450LM_{4a}	1.6	—	—	—	—
P-450LM_{4b}	0.40–1.1	2.30	0.2	6β	0.012

[a] Data from Klotz et al. (*16,36*). Activities are AHH, aryl hydrocarbon hydroxylase; EROD, ethoxyresorufin *O*-deethylase; ECOD, ethoxycoumarin-*O*-deethylase.

[b] Data from Williams and Buhler (*37,38*). Assignments are based on assumption that LM_1 = DE_bHA_1; LM_2 = DE_cHA_1; LM_3 = DE_a; LM_{4a} = DE_cHA_3; and LM_{4b} = DE_cHA_2.

[c] ND = not detectable. Limits of detection in original references.

[d] Not assayed.

[e] 6β was the sole product of P-450A. Other products are primary but not the only metabolites formed.

[f] All trout isozymes were listed as forming 6β-, 7α-, and 16α-hydroxytestosterone, each at less than 0.003 nmole/min/nmole P-450. P-450 LM_{4a} activity was not reported.

Table 6. Benzo(a)pyrene metabolites formed by purified, reconstituted scup cytochrome P-450E.[a]

Metabolite	P-450E + epoxide hydrolase	
	pmole/nmole P-450/min	Percent of total
9,10-Dihydrodiol	124	42
4,5-Dihydrodiol	ND[b]	—
7,8-Dihydrodiol	100	34
1,6-Quinone	ND	—
3,6-Quinone	62	21
6,12-Quinone	ND	—
9-Hydroxy	8	3
1-, 3-, or 7-Hydroxy	ND	—

[a]Data from Klotz et al. (*36*).
[b]Not detectable (< 1 pmole/nmole P-450/min).

regulation of isozymes such as cytochromes P-450E or P-450 LM_{4b} in fish could be instrumental in understanding the activation of environmental PAH carcinogens.

BNF-Inducible Isozymes

It is clear from Tables 4 and 5 that some of the scup and trout isozymes have similar physicochemical properties as well as similar catalytic functions. As pointed out earlier (*12*), scup cytochrome P-450E and trout cytochrome P-450 LM_{4b} share many properties and appear to be related enzymes. Properties established thus far for cytochrome P-450c from cod and DBA-P-450-I from little skate are similar to those of cytochrome P-450E and cytochrome P-450 LM_{4b}. These forms are compared in Table 7.

Cytochrome P-450E was initially purified from fish, sampled directly from the environment, which had very high levels of EROD and AHH activities. Earlier studies had shown that liver of these fish also had large amounts of a microsomal protein with M_r of 54,000, the same molecular weight as that protein appearing in scup induced with 3-MC (*25*). Those properties seen in 3-MC-induced scup liver microsomes are characteristic of purified cytochrome P-450E (*25,36*). Trout cytochrome P-450 LM_{4b} and cod cytochrome P-450c were both purified from BNF-treated animals. The fact that an isozyme is purified from a BNF-treated animal does not, in and of itself, establish that protein as one induced by BNF. However, these forms were not prominent in control animals, and their properties are like those induced in microsomes. Thus, the three forms in these species appear to have similar regulation as well as physicochemical and catalytic properties. Based on its properties and inducibility by a polynuclear aromatic hydrocarbon, we conclude that fraction DBA-P-450-I from little skate contains an isozyme that is an elasmobranch counterpart to these teleost isozymes. Furthermore, it is evident from Table 7 that these fish enzymes have properties similar to those of rat cytochrome P-450c, the major BNF-inducible isozyme in that mammalian species.

Table 7. Properties of apparent hydrocarbon-inducible cytochrome P-450 isozymes from fish.

Property	Scup P-450E[a]	Trout P-450 LM_{4b}[b]	Cod P-450c[c]	Skate P-450 I[d]	Rat P-450c[e]
Fe^{2+}–CO λ_{max}	447	447	448	448	447
M_r, kD	54.3	58.0	58.0	B[f]	56.0
AH hydroxylase activity, P-450 nmole/min/nmole	0.9	0.4–1.1	—	0.8	19.2
ER *O*-deethylase activity, P-450 nmole/min/nmole	7.1	2.3	1.07	—	5.3
Acetanilide hydroxylase activity, P-450 nmole/min/nmole	0.43	0.53	—	—	—
10^{-4}M ANF-inhibition[g]	80–90%	70–90%	—	90%	90%
Active inducers	3-MC, BNF, PCB	BNF, PCB	BNF	DBA	3-MC, BNF, PCB

[a] Data from Klotz et al. (*16,36*) and Kloepper-Sams et al. (*42*).
[b] Data from Williams and Buhler (*37,38,43*) Assignments are based on assumption that $LM_1 = DE_bHA_1$; $LM_2 = DE_cHA_1$; $LM_3 = DE_a$; $LM_{4a} = DE_cHA_3$; and $LM_{4b} = DE_cHA_2$.
[c] Data from Goksøyr (*39*).
[d] Data from Ball et al. (*35*).
[e] Data from Ryan et al. (*22*).
[f] Not assayed.
[g] Inhibition of reconstituted AHH activity.

Immunochemical Studies

Immunochemical studies have proven to be extremely useful in establishing structural homologies between cytochrome P-450 isozymes. Polyclonal antibodies have now been prepared against scup cytochrome P-450E (*19,42,44*), against trout cytochrome P-450 LM_{4b} (*38*), and against cod cytochrome P-450c (*39*). Recently, we have also described a library of monoclonal antibodies to scup cytochrome P-450E (*45*). Studies (*42,44*) with both the polyclonal and monoclonal antibodies prepared against cytochrome P-450E have established that this isozyme is the major and apparently only form induced in scup by BNF, polynuclear aromatic hydrocarbons, or polychlorinated biphenyls. Likewise, studies with antibodies prepared against trout cytochrome P-450 LM_{4b} and cod cytochrome P-450c have shown that these proteins are major BNF-inducible, and in trout PCB-inducible, isozymes in these species (*38,39,43*). Furthermore, studies in progress indicate extensive cross-reactivity between these isozymes. The precise degree of relationship is not yet established, although the strength of cross-reactivity in immunoblot analyses indicates a close relationship among these three proteins.

The relationship between some of these fish proteins and the major form induced by BNF in mammalian species has been evaluated with polyclonal and monoclonal antibodies prepared against both the mammalian and fish isozymes. Of nine monoclonal antibodies against scup cytochrome P-450E, one strongly and specifically cross-reacts with cytochrome P-450c from rats (*42*). In a reciprocal study using monoclonal antibodies prepared against rat cytochrome P-450c, two of ten monoclonals

specific to this rat isozyme also weakly recognized scup cytochrome P-450E, but not other proteins in scup microsomes (*16*). The results demonstrate that scup cytochrome P-450E is immunochemically related to cytochrome P-450c from rats. However, the facts that only one of nine monoclonals against scup P-450E strongly recognized rat P-450c, and that only two of ten against P-450c recognized P-450E, indicate that the two proteins do not share extensive structural homology. Consistent with this conclusion, the NH_2-terminal amino acid sequence of scup cytochrome P-450E shows no homology with the NH_2-terminal sequence of rat cytochrome P-450c (*36*). Moreover, although polyclonal anti-P-450E inhibits EROD activity in teleost microsomes, it does not inhibit EROD activity of BNF-induced rat liver microsomes (*19*). Studies by Williams and Buhler demonstrating that polyclonal anti-rat P-450 BNF-B did not inhibit catalytic activity of trout cytochrome P-450 LM_{4b} (*39*), also suggest little immunochemical relationship between cytochrome P- 450c and the BNF-induced fish isozymes. Nevertheless, the results with the monoclonal antibodies do show the presence of a least one highly conserved epitope in scup cytochrome P-450E and rat cytochrome P-450c, which we have concluded (*42,44*) reflects the presence of some highly conserved sequence.

Based on the apparent relationships between the BNF-induced forms in various teleosts, the above-indicated relationship between scup cytochrome P-450E and rat cytochrome P-450c, and the demonstrated homology between rat cytochrome P-450c, rabbit cytochrome P-450 form 6, and mouse cytochrome P_1-450 (*34*), we propose that these various fish and mammalian isozymes are counterparts in these species (Table 8). The fish and mammalian forms might be considered to be orthologous proteins (*46*). Whether the fish BNF-induced forms are also related to mammalian cytochrome P-450d is not known, but based on the relationship between P-450c and P-450d, could be expected (*47,48*).

Other Cytochrome P-450 Forms

The major PAH or BNF-inducible cytochrome P-450 isozymes have received the greatest attention in aquatic species. However, several other forms purified from fish or invertebrates have properties that are of substantial interest.

Table 8. Proposed homologous forms of teleost and mammalian BNF-inducible cytochrome P-450.

Species	Isozyme designation
Fish	
Scup	P-450E
Rainbow trout	P-450 LM_{4b}
Cod	P-450c
Mammals	
Rat	P-450c
Rabbit	P-450 form 6
Mouse	P_1-450

Cytochrome P-450 LM_2 from rainbow trout is a form that has been demonstrated (*49,50*) to combine two very important functions. First, this form very efficiently activates the mycotoxin aflatoxin B_1 to the 2,3-epoxide (*49*), the most carcinogenic metabolite of this compound. Accordingly, the well-known initiation of aflatoxin B_1 carcinogenesis in rainbow trout could depend on the function of cytochrome P-450 LM_2. Second, this isozyme also appears to efficiently catalyze the ω-1 hydroxylation of lauric acid (*50*), which occurs as a dominant pathway in trout. The combination of functions in chemical carcinogenesis and in endogenous substrate metabolism by this isozyme indicates further work would be fruitful.

Cytochrome P-450A from scup liver has been identified as a major testosterone 6-hydroxylase in this species (*16*). This isozyme possesses unique requirements for conspecific (i.e., scup) cytochrome P-450 reductase and conspecific cytochrome b_5 in reconstitution of its known catalytic functions. The identity of mammalian homologs to cytochrome P-450A is uncertain. Rat cytochrome P-450 PB-2a (*27*) is a prominent androstenedione 6β-hydroxylase and may be related to the scup cytochrome P-450A. However, rat P-450 PB-2a is induced by PCN and the inducibility of any counterpart isozyme in teleost species has not been demonstrated.

Many of the characteristics of scup cytochrome P-450A parallel those of rat cytochrome P-450d (ISF-G), the less abundant 3-MC-inducible form that is also strongly induced by isosafrole (*24*). Cytochrome P-450d is closely related immunochemically and in primary sequence to the major 3-MC-inducible form, cytochrome P-450c (*48,51*). As we have found that cytochrome P-450A is not immunochemically related to cytochrome P-450E (*16*), it is unlikely to be a counterpart of cytochrome P-450d. Whether cytochrome P-450 gene families of coordinately regulated members, like the rat 3-MC family containing cytochromes P-450c and P-450d, occur in fish is an open question. It is possible that gene families exist in teleost species but that they may be represented by fewer members than in the more recently evolved higher vertebrates.

Two forms with very similar properties have been purified from BNF-treated trout; cytochrome P-450 LM_{4b}, the most abundant, and cytochrome P- 450 LM_{4a}, a less abundant form. These two proteins are distinguished by chromatography, but are indistinguishable on the basis of molecular weight, optical properties, amino acid composition, catalytic properties, or immunochemical properties (*48*). A similar phenomenon has been reported in rats by one group (*52*) who purified proteins termed 3-MC1 and 3-MC2 that were distinguished by chromatography (HPLC) but identical in other properties including catalytic function and NH_2-terminal sequence. Both 3-MC1 and 3-MC2 appear to be equivalent to cytochrome P-450c. The precise relationship between these chromatographically separable but otherwise identical forms in rat or in fish remains to be established.

In addition to these fish forms, several fractions have been resolved from spiny lobster hepatopancreas by

James and Shiverick (*53*). The most highly purified form, designated fraction D1, has a molecular weight of 52,500. This form catalyzes an extremely efficient 16α-hydroxylation of progesterone, about 50 nmole/min/nmole P-450 and efficient 6β- and 16α-hydroxylation of testosterone. Homology with other forms has not been established.

Endogenous Regulation

Induction of microsomal monooxygenase activity by the chemical environment can be interpreted as due to expression of genes for PAH- or BNF-inducible isozymes, such as those described above. The regulation of microsomal activities by biological variables is not as well understood. As considered in a recent review (*27*), patterns of regulation by hormonal factors are of major interest. Strong similarities exist in cytochrome P-450 function between some mammalian and aquatic species. In the liver of both mammals and fish (*12*), there are generally lower levels of cytochrome P-450 and lower catalytic efficiencies with some substrates in reproductively active females than there are in males. There are, however, some important distinctions in this pattern between the two vertebrate groups.

The magnitude of the sex differences found in fish liver is often substantially greater than that seen in mammalian systems. In several marine fish species the levels of cytochrome P-450 and catalytic function with some xenobiotic substrates are 10-fold greater in seasonally mature or ripe males than in seasonally mature females (*54*), far greater than any such difference seen in mammals. Not only the magnitude, but also the mechanisms involved in producing this sex difference could differ between fish and mammals. Estradiol has been demonstrated to suppress the levels of hepatic cytochrome P-450 and some catalytic functions in some fish (*55,56*) and could be the major effector of sex differences in fish. In rats, testosterone is usually recognized as a major effector (*57*). The mechanisms by which estradiol exerts this effect in fish and the identity of isozymes that are primarily regulated in this sex difference, are not known. Whether the pituitary is a required participant in this action as it is in the rat response to testosterone (*58*) is also not clearly established (*56*). Identifying cytochrome P-450 isozymes that are sexually differentiated in fish liver and comparing these with sexually differentiated forms in mammals could indicate the general significance of such sex differences in vertebrate species. In this regard, sexual differentiation of cytochrome P-450 LM_2 in rainbow trout kidney has recently been described (*59*). As with functions in the liver, the mechanism of this cytochrome P-450 LM_2 regulation in kidney is not known.

Developmental status and hatching have also been shown to regulate the function of microsomal cytochrome P-450 in fish with patterns that are reminiscent of those seen during mammalian development and parturition (*60,61*). Neither the mechanisms of regulation during development nor the isozymes regulated have been established. However, the fact that AHH activity shows a developmental pattern suggests that a counterpart to scup cytochrome P-450E is among forms regulated in those species studied, the killifish, *Fundulus heteroclitus*, and brook trout, *Salvelinus fontinalis*. A counterpart to cytochrome P-450E could also be the AHH catalyst that is inducible by PCBs in embryonic stages of fish (*62*).

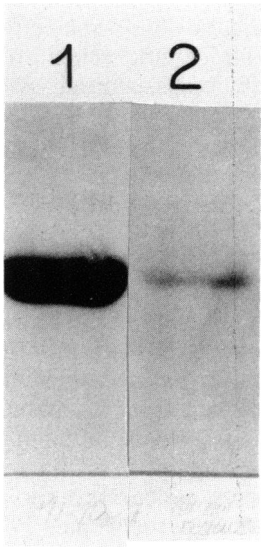

FIGURE 1. Immunoblot of deep sea fish liver microsomes with anti-scup P-450E. Microsomal protein containing 20 pmole P-450/lane was separated as before (*65*). Lane 1, Hudson Canyon fish; EROD, 2.0 nmole/min/nmole P-450. Lane 2, Carson Canyon fish; EROD, 0.2 nmole/min/nmole P-450. From Stegeman et al. (*65*).

Environmental Induction

A major concern in aquatic toxicology is the estimation and interpretation of effects in natural populations. Previous studies have shown that some untreated fish sampled directly from the environment have high levels of monooxygenase activities (*25*). The causes of such apparent induction have not been explicitly demonstrated, but association with levels of hydrocarbons in the environment has been made in several such studies (*63,64*). More recently, antibodies to scup cytochrome P-450E have been employed to demonstrate the presence of a counterpart to this hydrocarbon-inducible isozyme in fish from the deep sea (Fig. 1) (*65*). An association between the amounts of cytochrome P-450E counterpart and the levels of specific chlorobiphenyl isomers in the liver of those fish (*65*) demonstrated the usefulness of immunological techniques to identify animals environmentally exposed to biochemically significant levels of MC-type inducers.

However, the general significance of environmental induction is not yet known. Increased tolerance to pollutants could result from enhanced elimination due to induction. On the other hand, the proposed role of hydrocarbon-induced isozymes in activation of some PAH

carcinogens could have deleterious effects. Establishing the mechanisms underlying other effects or biological changes that might be associated with induction of cytochrome P-450 forms would be of great value in assessing the significance of environmental induction.

Conclusion

Cytochrome P-450 isozymes in fish include a counterpart to the major BNF-inducible form in mammals. This form could play the dominant role in metabolism of many PAH. Studies of this and other isozymes may define the species, organ and/or cellular susceptibility to chemicals in the aquatic environment. The similarities and distinctions that are being discovered between fish and mammals suggest fruitful avenues for study of fundamental aspects concerning the function, regulation, and evolution of cytochromes P-450.

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