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Hepatic Microsomal Epoxide Hydratase of Bluegill

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SUMMARY: Hepatic microsomal epoxide hydratase of the bluegill fish shows characteristics similar to those of the marine fish. The bluegill hepatic microsomal epoxide hydratase activity towards styrene oxide is higher (4n-mole/min per mg protein) and that of mixed-function oxidase towards aldrin epoxidation is lower (0.7n-mole/min per mg protein) than the corresponding enzymes of the male mouse (1.90 and 2.0n-mole/min per mg protein, respectively, for epoxide hydratase and aldrin epoxidase).

Hepatic microsomal mixed-function oxidase (MFO) of mammals (1-5), birds (6-8) and fish (9-13) can epoxidize aliphatic and aromatic hydrocarbons (polycyclic aromatics, drugs, pesticides, steroids, etc). These epoxides can be metabolized further by the hepatic microsomal epoxide hydratase (EH) (5) and/or soluble glutathione S-epoxide transferase (13). EH has been well characterized and even purified in several mammalian species (5,14). Among fish, this enzyme has been studied only in marine species (12,15,16). The activity of EH towards styrene oxide appears to be optimum at temperatures between 40° to 45°C in flounder and skate from coastal Maine and 45° to 50°C in sheepshead from coastal Florida. The optimum pH lies between 8 and 9.5. In these species the levels of EH activity range from: 0.4 to 7.6n-mole styrene glycol formed per min per mg microsomal protein (12) and from 0.051 to 8.86n-mole cyclodiene epoxide disappeared per min per mg protein (16). This enzyme has not been studied in freshwater fish (17). We have been studying the epoxidation and hydroxylation of cyclodiene insecticides in freshwater fish (9-11). Since cyclodiene epoxides are metabolized *in vivo* by freshwater fish to *trans*-diols (18) such hydroxylations may be catalyzed by EH. We have therefore characterized this enzyme in freshwater bluegill

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(Lepomis macrochirus) using styrene oxide as a substrate to use this as a model for studying hydroxylation of cyclodiene epoxides. Preliminary results are presented.

MATERIALS AND METHODS:

Bluegill provided by Illinois Hatchery (Spring Grove, IL) weighed 44 to 64 gm per fish, while those from McGraw Wildlife Foundation (East Dundee, IL) weighed 94 to 100 gm. These were maintained at John G. Shedd Aquarium, Chicago and sacrificed within half hour of their transfer to the laboratory. [7-^{3}H]styrene oxide (27mci/mmol) purchased from Amersham Searle, Arlington Hts., IL, was analytically pure as checked by thin-layer chromatography followed by X-ray autoradiography (19). Livers were excised and homogenized in ice-cold 0.25 M sucrose (10% w/v). The homogenate was centrifuged at 10,800 g for 15 min and the supernatant spun at 105,000 g for 90 min. When calcium aggregation was used, 1 ml of 26 mM calcium chloride was added to 33 ml of the mitochondrial-supernatant by stirring continuously for 10 min (8,10,20). This was centrifuged at 35,000 g for 20 min to obtain microsomes. The microsomal pellet obtained by either method was resuspended in the same volume of 0.25 M sucrose and spun again at the previous speed but for half of the previous spinning time. The washed microsomal pellet was resuspended in 0.25 M sucrose.

Enzyme assays were carried out by incubating 2 mM styrene oxide with 1 mg of microsomal protein at 37°C for 15 min (21,22). 0.5 ml of the incubation mixture contained 0.125 ml of 0.5 mM Tris buffer containing 0.1% Tween-80 at pH 9.0. The reaction was stopped by adding petroleum either and freezing the incubate on dry ice. After aspirating the pet. ether the glycol was extracted with 2 ml of ethyl acetate. 0.2 ml aliquots of the latter were counted radiometrically (19,22). All incubations were carried out in triplicates and each experiment repeated at least twice. Average values of these experiments were used.

For comparative purposes similar assays were used for 4-month old mice (Swiss Webster, ICR strain) and frog Xenopus laevis (NASCO, Fort Atkins, Wis.). Protein determinations were carried out by a modification of Lowry's method (23).

RESULTS:

The activity of the bluegill microsomal EH showed maximum levels alkaline range, around 9 pH (Fig. 1B) when incubations were carried out at 37°C for 30 min. This appears similar to the reports with marine fish (22,24) and mammals (1-5,21). This activity is confined mostly in the microsomal subcell fraction. The activity at pH 9.0 (at 37°C) was linear up to 60 min. This was: 15 min = 6.22, 30 min = 10.21, 60 min = 22.51n-mole styrene glycol formed per mg protein. Under these conditions the linearity was maintained over a wide range of temperature i.e. 18° to 50.5°C and started declining thereafter (Fig. 1C). Similar insensitivity of EH to temperatures of 45° to

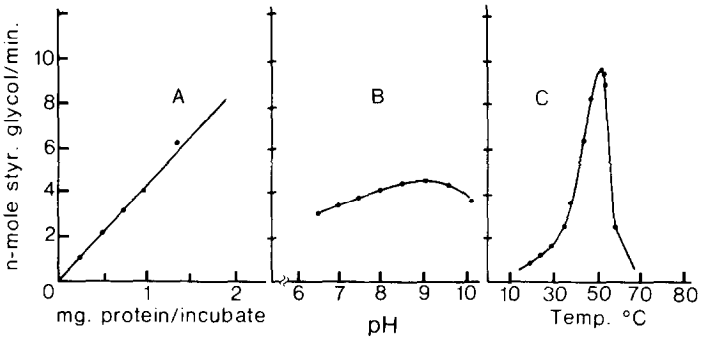

Fig. 1. Effect of microsomal protein concentration (A), pH (B) and temperature (C) of incubation mixture on EH activity towards [7-^{3}H]styrene oxide. The units of activity are on: A = per incubate and B and C = per mg microsomal protein basis.

50°C has been observed in marine fish (22,24). That the formation of styrene glycol was enzymatic was checked by increasing enzyme concentration which showed a linear relationship between product formation and protein concentration (Fig. 1A). Thin-layer chromatography using 0.25 mm silica gel G F-254 plates developed in chloroform-methanol (1:1) showed the product to be styrene glycol (Rf values: styrene oxide 0.65, [7-^{3}H]styrene oxide 0.62, styrene glycol 0.17) as checked by ultraviolet viewing and X-ray autoradiography (19).

Incubating styrene oxide at concentrations ranging from 0.02 to 2.0 mM with 1 mg of microsomal protein (37°C, 15 min) gave Km values (using Lineweaver-Burke plots) ranging from 0.26 to 0.32 mM styrene oxide and Vmax values between 7.70 and 8.33n-mole styrene glycol formed/min per mg protein.

Calcium aggregation seems to yield microsomes having the specific activity same as those obtained by conventional methods (Table 1). Freezing the microsomes at -20°C for 6 weeks lowered the specific activity by only 12% i.e. from 3.78 to 3.33n-mole/min per mg protein. A comparison of aldrin epoxidase by MFO and styrene oxide hydratase by EH of bluegill, Xenopus and mouse shows that the two aquatic species have lower levels of activity of MFO but 2 to 4 times higher of EH (Table 1). Mice, on the other hand, show higher MFO activity toward aldrin epoxidation but slightly

Table I. Styrene oxide hydrase and aldrin epoxidase activity of hepatic microsomes of bluegill, Xenopus and mouse.

Species[b]	n-mole product[a]/min per mg protein	
	Epoxide hydratase	MFO aldrin epoxidase
Bluegill	3.23,3.31,3.54,4.20(4.62)	0.70
Xenopus	0.45,0.49(0.11,0.22)	0.30,0.32,0.36
Mouse	1.90(1.26)	2.05

[a] = styrene glycol for EH and dieldrin for MFO (data from references 9-11 and 25)
[b] = values in parentheses: bluegill-fry (2 to 4.4 g), frog = at 25°C, mouse = females

lower activity of EH. A similar relationship between aldrin epoxidation (16) and cyclodiene epoxide (16) or styrene oxide hydration (12) has been reported in marine fish. For example, in sheepshead, mullet and sting ray MFO epoxidase activity was .213, .151 and .010, respectively. While EH activity levels toward cyclodiene epoxide were 2.25 for sheepshead (6.1 with styrene oxide), .90 for mullet, and 8.86 (7.6 with styrene oxide) for sting ray (12,16). The optima for epoxidase and EH are 7.4 and 9.0, respectively (12,16), so the lower levels of former may not be due to further and faster hydration of the epoxide. Also, the low MFO levels in fish appear to correlate with the cytochrome P-450 concentration (12,16). Since the recoveries of added radioactivity are better than 95 per cent, the binding of the epoxide to EH to make it unextractable may not account for the observed differences.

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