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MICROSOMAL AND CYTOSOLIC EPOXIDE HYDROLASE IN *DROSOPHILA MELANOGASTER*

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Abstract—Subcellular fractions from *Drosophila melanogaster* and rat liver were investigated on their epoxide hydrolase activity Both microsomes and the post-microsomal supernatant of *Drosophila* appeared to contain epoxide hydrolase activity using styrene-7,8-oxide as the substrate Based on body weight, these activities were in the same order of magnitude Rat liver cytosol was able to catalyze the hydrolysis of styrene oxide only if the glutathione *S*-transferase activity was blocked

Epoxides are an important class of chemical compounds with respect to their potential reactivity towards cellular nucleophiles, which may initiate carcinogenic and mutagenic processes [1]. Exposure of humans is possible because some of the epoxides are used industrially in large volumes. However, the organism itself is able to generate epoxides from aromatic and olefinic xenobiotics by biotransformation involving the cytochrome P-450 mediated oxidative pathway [2]. In addition to the potency to produce adducts with cellular macromolecules, epoxides can be detoxified either by non-enzymatical rearrangement to phenolic and analogous compounds, by glutathione conjugation or by enzymatically catalyzed addition of water. The latter pathway involves epoxide hydrolase (EC 3.3.2.3), an enzyme known to be present in several cellular compartments [3, 4].

With respect to the genotoxic risk presented by epoxides and other electrophilic agents, many short-term tests are presently in use assessing mutagenic potency of such chemicals [5] Among these assays the fruitfly *Drosophila melanogaster* establishes a genetically well-defined indicator organism, capable of detecting a wide array of pre-mutagens and pre-carcinogens [6–8]. As the ultimate fate of epoxides in an organism will largely determine their potential harm, knowledge of the enzymes involved in their biotransformation is obviously needed. Studies from this laboratory [9–14] as well as from others [15–17] have already established the presence of some xenobiotic-metabolizing enzyme activities including epoxide hydrolase in *Drosophila*. Investigations on the *Drosophila* glutathione *S*-transferase indicated that this enzyme system is unable to conjugate a number of epoxides in the way it does in mammals [18]. The present report focuses on epoxide hydrolase mediated detoxication of epoxides in *Drosophila*, with emphasis on its intracellular localization, in comparison with the enzyme in rat liver

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MATERIALS AND METHODS

Chemicals Styrene-7,8-oxide (STOX) was obtained as an analytical grade product from Merck-Schuchardt (Darmstadt, G.F.R.); diethylmaleate was produced by Aldrich Chem. Co. (Milwaukee, U.S A), and *p*-nitroanisole and *n*-butylboronic acid were products of Fluka AG (Buchs, Switzerland) Styrene diol (1-phenyl-1,2-ethanediol) was synthesized as reported before [10]. All other chemicals were of the best quality commercially available. Water was glass-distilled prior to use.

Animals. Male rats of the laboratory-bred SPF Wistar strain weighing 180–200 g were used for rat liver experiments They were fed a commercially available diet (Hope Farms, Woerden, The Netherlands). Food and tap water were allowed *ad libitum* *Drosophila melanogaster*, strain Berlin K was obtained from the department of Radiation Genetics and Chemical Mutagenesis, Faculty of Medicine, University of Leiden. They were provided as adult flies from mass cultures that were reared at 25°

Subcellular fractionation. Rats were starved overnight and killed by cervical dislocation. The livers were perfused *in situ* via the portal vein with 0 13 M sodium phosphate buffer pH 7 4, removed, washed, minced and homogenized in a Potter–Elvehjem tube with a Teflon pestle The homogenate was centrifuged for 20 min at 20,000 *g* in a Beckman JA-20 centrifuge. The supernatant was filtered through tissue paper to remove floating fat and further centrifuged for 75 min at 100,000 *g* in a Beckman L5-50E ultracentrifuge. The microsomal pellet thus obtained was washed, centrifuged again and finally suspended in the buffer mentioned before Flies were killed by freezing and processed as described for rat liver All manipulations were performed at 4° Protein contents of 100,000 *g* supernatants (cytosol) and microsomal suspensions were determined according to Lowry *et al* [19], using bovine albumine as the standard

Enzyme assay Epoxide hydrolase activity was assayed using STOX as the substrate in concentrations up to 1 mM Incubations took 5 min for cytosolic, and 15 min for microsomal activity, in

0 13 M sodium phosphate buffer pH 7 4, at 27° and 37° for *Drosophila* and rat liver enzymes, respectively In general, the protein content was 0 5 mg/ml incubation mixture Conditions were chosen in order to obtain linearity in product formation with respect to time and protein concentration When rat liver cytosol was used as the enzyme source, 2 mM diethylmaleate was added to the incubation mixture to inhibit glutathione conjugation Microsomal contamination of cytosolic preparations was controlled by determining benzo(*a*)pyrene hydroxylation as reported by Yang and Kicha [20], adopted for insect enzymes by Baars and Driessen [21]

Styrene diol was measured by a modification of the method described by Belvedere *et al* [22]: the diol produced was extracted twice from the incubation mixture with diethylether, and the organic phase was removed and allowed to evaporate overnight at room temperature, adding 0.5 ml ethanol to avoid total dryness The residue was dissolved in 0 4 ml acetone to which 10 μl of a *n*-butylboronic acid solution in dimethylformamide (25 mg/ml) was added to derivatize the diol Aliquots were analysed gaschromatographically as described before [10], *p*-nitroanisole was used as the internal standard

Appropriate control experiments were done to correct for any non-enzymatic formation of styrene diol

Apparent enzyme kinetic constants were calculated from V vs S plots using a computer program based on the statistical analysis of Wilkinson [23]

RESULTS AND DISCUSSION

First attempts to estimate rat liver cytosolic epoxide hydrolase with STOX as the substrate were unsuccessful Although styrene diol was assayed as the reaction product, alterations by glutathione could not be excluded, as rat liver cytosol contains considerable amounts of glutathione and glutathione *S*-transferase, able to remove significant amounts of STOX from the incubation mixture [24]. In successive investigations the glutathione conjugation pathway was blocked by binding endogenous glutathione with an appropriate amount of diethylmaleate prior to the addition of STOX to the incubation mixture Under these conditions a small but significant cytosolic epoxide hydrolase activity could be measured, while glutathione *S*-transferase activity was no longer detectable, neither with STOX nor with 1-chloro-2,4-dinitrobenzene as the substrate In rat liver microsomes, glutathione *S*-transferase activity towards STOX as the substrate could not be demonstrated, as already reported earlier [25], while we recently described the apparent lack of glutathione-epoxide conjugating activity in *Drosophila* [18, 26].

Table 1 Specific activities of *Drosophila melanogaster* and rat liver epoxide hydrolase, for styrene oxide as the substrate

	Microsomes	Cytosol
Drosophila	42 5 ± 4 1 (6)	38 7 ± 3 2 (5)
Rat liver	13 6 ± 0 6 (4)	2 5 ± 0 1 (5)

Data are presented as nmoles of styrene diol produced/min per g body wt (mean ± S E M), number of experiments in parentheses

The specific epoxide hydolase activities as determined in the cytosolic and microsomal fractions of *Drosophila* and rat liver are given in Table 1 In this table the activities are based on body weights as insects lack a specific organ for metabolizing foreign compounds whole body homogenates were used as the starting material. Rat liver data were extrapolated to total body weight (ignoring activities in other body parts [27]) in order to make a comparison possible *Drosophila* epoxide hydrolase is about as active in cytosol and microsomes (Table 1). Rat liver is much less active. microsomes contain about 1/3, and cytosol 1/15 of the corresponding *Drosophila* activity

Enzyme kinetic constants of the enzymes are presented in Table 2. Obviously rat liver enzymes have a greater affinity for STOX compared with the *Drosophila* enzymes. The observed differences in V_{max} values are not remarkable in view of the specific function of the rat liver in metabolizing xenobiotics

Figure 1 depicts the relation between enzyme activity per mg of protein and the amount of protein in both subcellular compartments, for *Drosophila* per g body wt, and for rat liver per g liver wt On this basis, rat liver microsomes have by far the greatest capacity to hydrolyze STOX

Microsomal epoxide hydrolase in *Drosophila* had been described previously [10, 13], but the presence of a soluble epoxide hydrolase was not excluded With respect to mammals, the conversion of an aromatic epoxide to the corresponding dihydrodiol was reported already by Jerina *et al* [28], but most attention has been focused on the microsomal enzyme [3, 4] More recently however, a number of reports

Table 2 Enzyme kinetic constants of *Drosophila melanogaster* and rat liver epoxide hydrolase for styrene oxide as the substrate

			V_{max} (nmol/min/mg protein)	K_m (mM)
Drosophila	Microsomes	(6)	6 06 ± 0 58	0 36 ± 0 07
	Cytosol	(5)	0 70 ± 0 06	1 43 ± 0 25
Rat liver	Microsomes	(4)	25 46 ± 1 05	0 069 ± 0 014
	Cytosol	(4)	1 41 ± 0 09	0 040 ± 0 003

Data are presented as mean ± S E M , number of experiments in parentheses

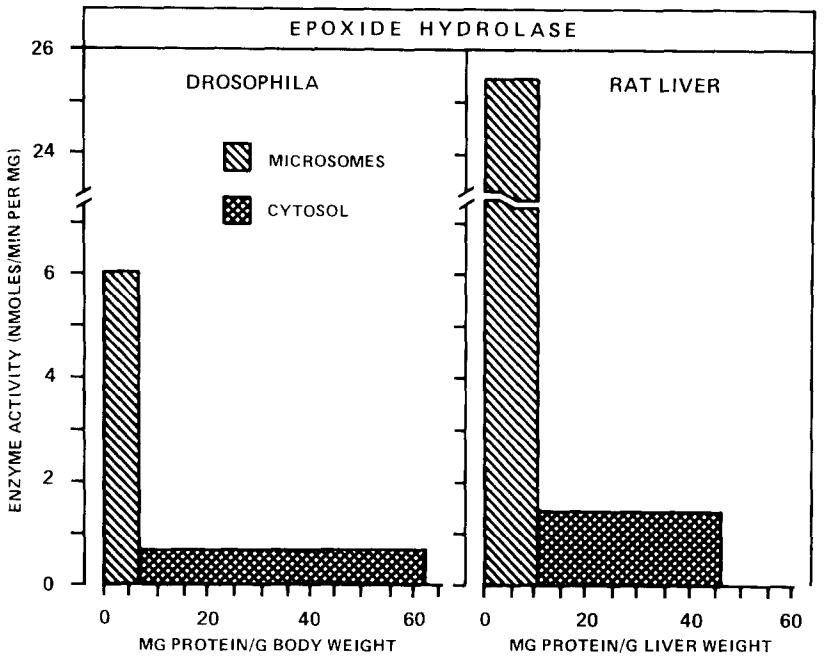

Fig 1 Histogram of the distribution of epoxide hydrolase activity (using styrene-7,8-oxide as the substrate) between microsomes and cytosol of *Drosophila melanogaster* and rat liver, respectively

described epoxide hydrolase activity in the soluble fraction of mammalian livers [29, 30]

The present study demonstrates such a soluble epoxide hydrolase activity in *Drosophila* Its relative activity towards STOX as the substrate of choice, compared with the microsomal enzyme, is greater than in rat liver However, the fourfold difference in the apparent K_m values suggest a distinct substrate specificity, in accordance with similar findings on the enzymes in rat and mouse liver [31, 32] When the results are taken together with the findings on glutathione *S*-transferase in *Drosophila* [18, 26], it is obvious that the presence of a considerable amount of cytosolic epoxide hydrolase (based on body weight) compensates at least partly for its lack of glutathione *S*-transferase activity towards epoxides

In any genotoxicity assay the end-points observed following exposition to precarcinogenic or premutagenic chemicals will depend on the intrinsic activity of activating and inactivating enzymes in that model With respect to *Drosophila melanogaster* the present results once more stress the necessity for a careful consideration of the metabolic fate of the particular chemical under test

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