

Characterization of Cytosolic Epoxide Hydrolase in Mouse Liver*

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In recent years it has become clear that many of the toxic, mutagenic and carcinogenic effects of xenobiotics are not due to the parent substances themselves. In many cases reactive intermediates, often formed *via* the cytochrome P-450 system, are directly responsible for these deleterious effects. One common type of reactive intermediate formed, for example, during the metabolism of polycyclic hydrocarbons by the cytochrome P-450 system, is epoxides. Such epoxides can generally be metabolized further to relatively harmless products by the phase II drug-metabolizing enzymes epoxide hydrolase(s) (E.C.3.3.2.3) and glutathione S-transferase(s) (E.C.2.5.1.18).

Originally, it was believed that epoxide hydrolase(s) was localized solely on the endoplasmic reticulum in hepatocytes. However, Hammock and his coworkers^{1,2} demonstrated that there is also a cytoplasmic form of this enzyme. The microsomal epoxide hydrolase has received much attention and has been thoroughly characterized and purified to homogeneity.^{3,4}

Much less is presently known about the cytoplasmic enzyme and its properties. Until more is known, we will be unable to assess the role of this epoxide hydrolase in the metabolism of different xenobiotics and in protection against the toxic,

mutagenic and carcinogenic effects of reactive intermediate epoxides. The goal of the present investigation was to characterize the cytoplasmic epoxide hydrolase in a number of different ways and, in particular, to determine properties of this enzyme which can subsequently be used to design a purification procedure.

These experiments were performed using male NMRI, C57 black, and CBA mice (Anticimex, Stockholm, Sweden) weighing 20–30 g and given free access to food pellets and water until sacrifice by cervical dislocation. The gall bladder was removed and the liver homogenized in 4 volumes of 0.25 M sucrose using 4 up-and-down strokes of a Potter-Elvehjem homogenizer at 440 RPM. The resulting suspension was centrifuged at 10 000 *g* for 10 min and the supernatant from this step then centrifuged at 100 000 *g* for 1 h. The clear high-speed supernatant under the lipid layer, designated cytosol, was carefully sucked off and used in the experiments. The high-speed pellet, the so-called microsomes, was resuspended in 0.25 M sucrose and used for comparison with the cytosolic fraction in one experiment. Time studies performed with NMRI cytosol demonstrated that the epoxide hydrolase activity in this preparation is stable for at least several weeks at –20 °C, for at least 4–6 days at 4 °C and for at least 2 days at 20 °C.

Epoxide hydrolase activity was measured using either styrene oxide,⁵ *trans*-⁶ or *cis*-stilbene oxide⁶ as the substrate. Tritiated *trans*- and *cis*-stilbene oxides of very high specific radioactivity were synthesized according to Dr. B. D. Hammock, University of California at Davis (personal communication). Glutathione S-transferase activity was measured with 1-chloro-2,4-dinitrobenzene as the second substrate.⁷

The molecular weight of the cytosolic epoxide hydrolase was determined by ultrafiltration on a column of Sephacryl S-300 (Pharmacia, Uppsala, Sweden) (2.5 × 90 cm) at 4 °C. 3 ml of the cytosol fraction to which standard proteins had been added was applied to this column and eluted with 0.1 M potassium phosphate, pH 7.0, at a flow rate of 14 ml/h.

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Table 1. Epoxide hydrolase activity in microsomes and cytosol from male NMRI mouse liver.

Fraction ^a	Epoxide hydrolase activity ^b (nmol/min mg protein) with different substrates		
	styrene oxide ^c	<i>trans</i> -stilbene oxide ^d	<i>cis</i> -stilbene oxide ^e
Microsomes	5.25 ± 1.66	0.41 ± 0.24	1.96 ± 0.42
Cytosol	4.79 ± 0.06	1.39 ± 0.48	0.01 ± 0.002

^a Microsomes and cytosol were prepared as described in the text. ^b The values are means ± S.E.M. for three individual animals. ^c Assayed in 0.1 M potassium phosphate, pH 6.8, for the cytosol and in 0.1 M glycine, pH 9.0, for the microsomes.

^d Assayed at pH 6.8 in the same buffer described in c. ^e Assayed at pH 9.0 in the same buffer described in c.

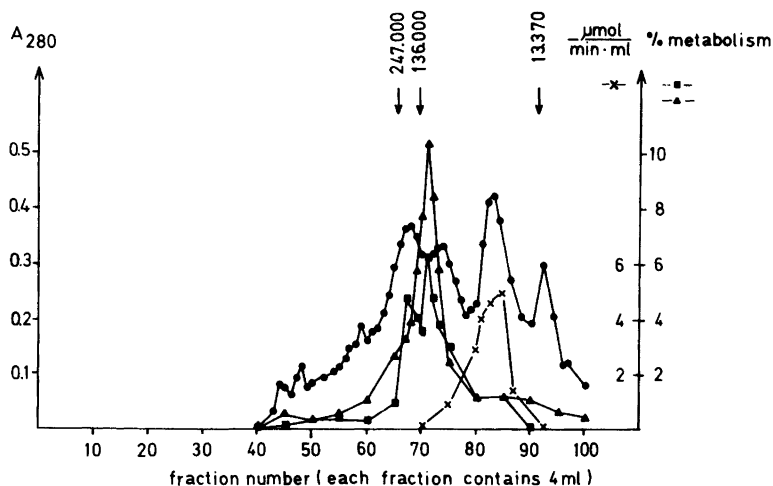


Fig. 1. Gel filtration of the cytosol fraction from mouse liver on Sephacryl S-300.

This experiment was performed as described in the text. The cytosol fraction from C57 black mice was used here, but similar results were obtained with NMRI and CBA mice as well. The symbols used are as follows: ■ = epoxide hydrolase activity measured using *trans*-stilbene oxide as substrate; ▲ = epoxide hydrolase activity with styrene oxide as substrate; × = glutathione *S*-transferase activity with 1-chloro-2,4-dinitrobenzene as substrate; ● = absorption at 280 nm. The elution positions and molecular weights of three standard proteins are indicated in the figure.

The isoelectric point was determined by applying 25 μ l of each cytosol fraction to commercially prepared slab gels (purchased from LKB, Bromma, Sweden) and running at 15 mA for 90 min. The enzyme was extracted from the gel with the appropriate buffer for enzyme assay at 4°C for several hours before being assayed.

Literature reports maintain that *trans*-stilbene oxide is a much better substrate for the soluble epoxide hydrolase than is styrene oxide,⁸ but this was not found to be the case in our hands. In the present experiments the cytosol fraction from NMRI mice hydrolyzed styrene oxide at a rate of 4.79 nmol/min mg protein, while the corresponding activity with *trans*-stilbene oxide was only 1.39 nmol/min mg protein (Table 1). It can also be seen from the table that the specific activity of styrene oxide hydrolase for microsomes and cytosol is nearly equal, whereas the cytosol hydrolyzes *trans*-stilbene oxide 3 times faster than the microsomes and the microsomes catalyze the hydrolysis of *cis*-stilbene oxide nearly 200 times more efficiently than the cytosolic fraction. Thus, *trans*- and *cis*-stilbene oxide would seem to be better substrates for distinguishing between the microsomal and cytosolic epoxide hydrolases than is the more commonly used styrene oxide.

As can be seen from Fig. 1, the cytosolic epoxide hydrolase — measured either with *trans*-stilbene

oxide or styrene oxide as substrate — demonstrates a molecular weight of approximately 130 000 upon gel filtration. This value agrees well with preliminary results published earlier.⁹ The presence of virtually all the epoxide hydrolase activity in a single peak suggests that there is only one form of this enzyme present in mouse liver cytoplasm. The activity peak contains no more than 20% of the total protein applied to the column, so this procedure may prove to be a valuable step in the future purification of the enzyme. It can also be seen from Fig. 1 that cytosolic glutathione *S*-transferase activity can be clearly separated from cytosolic epoxide hydrolase on the basis of molecular weight. The apparent molecular weight of the glutathione *S*-transferase(s) is about 50 000, which agrees with previous reports.¹⁰

As shown in Fig. 2, the isoelectric point of the cytosolic epoxide hydrolase in mouse liver — using either *trans*-stilbene oxide or styrene oxide as substrate — is approximately 5.6. Also in this case a single peak of activity is seen, indicating the presence of only one epoxide hydrolase. Again, cytosolic glutathione *S*-transferase activity is clearly separated from the cytosolic epoxide hydrolase, focussing as expected¹⁰ at a basic pH above 8, yet another strong piece of evidence that these two enzymes are different proteins.

Finally, the cytosolic epoxide hydrolase in mouse liver demonstrates a pH optimum of around 7.0 and

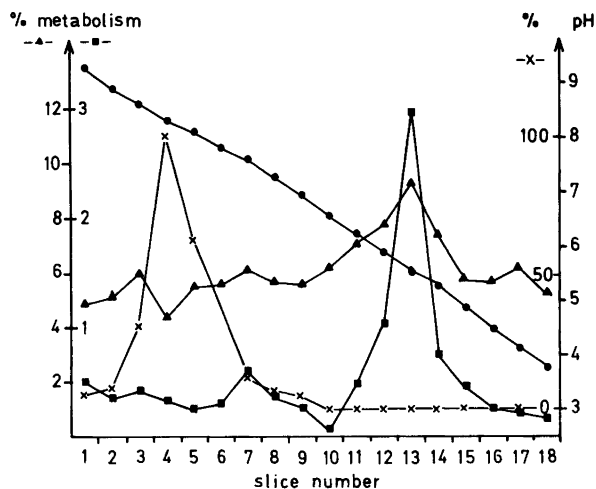


Fig. 2. Isoelectric focussing of the cytosol fraction from mouse liver.

This experiment was performed as described in the text. The cytosol fraction from NMRI mice was used here, but similar results were obtained with C57 black and CBA mice as well. The symbols used are as follows: ■ = epoxide hydrolase activity measured using *trans*-stilbene oxide as substrate; ▲ = epoxide hydrolase activity with styrene oxide as substrate; × = glutathione *S*-transferase activity with 1-chloro-2,4-dinitrobenzene as substrate; ● = pH.

distributes upon subcellular fractionation in the manner expected for a cytosolic enzyme. The next step in our investigations will be purification of this enzyme and subsequent further characterization.

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