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CHARACTERIZATION OF MULTIPLE EPOXIDE HYDROLASE ACTIVITIES IN MOUSE LIVER NUCLEAR ENVELOPE

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Abstract—A nuclear envelope-associated epoxide hydrolase in mouse liver that hydrates *trans*-stilbene oxide has been identified and characterized. This epoxide hydrolase is distinct from the enzyme in nuclear envelopes that hydrates benzo[*a*]pyrene 4,5-oxide and other arene oxides. This distinction was demonstrated by the criteria of pH optima, response to specific inhibitors *in vitro*, and precipitation by specific antibodies. The new epoxide hydrolase had a pH optimum of 6.8, was poorly inhibited by trichloropropene oxide, was potently inhibited by 4-phenylchalcone oxide, and did not bind to antiserum against benzo[*a*]pyrene 4,5-oxide hydrolase. This nuclear enzyme is similar in many of its properties to cytosolic and microsomal *trans*-stilbene oxide hydrolases and may be a nuclear envelope-bound form of these other epoxide hydrolases. It differed from these other *trans*-stilbene oxide hydrolases in that its affinities for both *trans*-stilbene oxide (measured as apparent K_m) and 4-phenylchalcone oxide (measured as I_{50}) were 4- to 20-fold lower than those of either the cytosolic or microsomal forms.

Epoxide hydrolases are a group of enzymes that play an important role in protecting the cell from the toxic and carcinogenic effects of a number of reactive epoxide intermediates [1–3]. The importance of this group of enzymes to the disposition and toxicity of these reactive epoxide intermediates has led to extensive investigation of the catalytic properties of epoxide hydrolases as well as the determination of their intracellular distribution. One major epoxide hydrolase, that enzyme responsible for the hydration of benzo[*a*]pyrene 4,5-oxide (BPO†) as well as many other polycyclic aromatic hydrocarbon-derived epoxides, has been shown to be located primarily in the membrane fractions of the cell. It is found at its highest concentration in the endoplasmic reticulum [2, 3], and to a lesser degree in the nuclear envelope [4–6]. In some tissues and species, this enzyme is found in the cytosolic fraction as well [7–10]. It is denoted here as "EH1". A second epoxide hydrolase that has been characterized more recently is responsible for the hydration of a number of alkene-derived and *trans*-disubstituted oxiranes such as *trans*-stilbene oxide (TSO) [11, 12], and is located primarily in the cytosolic fraction of the cell. This epoxide hydrolase has also been detected at lower concentrations in the mitochondria [13, 14], in peroxisomes [15], and in the endoplasmic reticulum fraction of certain tissues and species [16–18]. This epoxide hydrolase is referred to here as "EH2".

The endoplasmic reticulum has long been considered the major site of xenobiotic metabolism in the cell. It contains the bulk of the cytochrome P-450s in the cell and is also rich in such "phase II" enzymes as BPO hydrolase and UDP-glucuronosyltransferases [19]. More recent investigations have shown, however, that the cell nucleus may be considered as a major xenobiotic-metabolizing organelle as well. It contains all the enzymes necessary for multistep metabolism of foreign compounds, including cytochrome P-450 [20, 21], associated flavoprotein reductases [20, 21], and arene oxide hydrolases [4, 20, 21]. Because of the proximity of these nuclear enzymes to the ultimate target site of many reactive intermediates, chromosomal DNA, the role that they play in toxification and detoxification of reactive intermediates is doubtless very important to the well-being of the cell.

Earlier studies of BPO hydrolase activity in nuclear membranes showed that the properties of this enzyme could not be distinguished from those of its endoplasmic reticulum-bound counterpart. These studies concluded that, like cytochrome P-450, this epoxide hydrolase exists in forms that are both microsomal and nuclear, and that these corresponding forms appear to be identical [5, 6]. The present study investigates a second form of nuclear epoxide hydrolase; this form corresponds to the so-called "cytosolic" epoxide hydrolase that has been detected in the cytosolic cell fraction [11, 12, 22], the mitochondrial lumen [13, 14], and at lesser concentrations

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† Abbreviations: BPO, benzo[*a*]pyrene 4,5-oxide; TSO, *trans*-stilbene oxide; 4-PCO, 4-phenylchalcone oxide; TCPO, trichloropropene oxide; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; MES, 2-[*N*-morpholino]ethanesulfonic acid; TRIS, tris(hydroxymethyl)-aminomethane; HPLC, high pressure liquid chromatography; GARGG, goat antiserum to rabbit gamma globulin; EH1, the epoxide hydrolase that specifically hydrates BPO and other arene oxides; EH2, the epoxide hydrolase that specifically hydrates TSO and other *trans*-disubstituted oxiranes; I_{50}, the concentration of inhibitor *in vitro* that results in 50% inhibition of enzyme activity; K_m, the Michaelis constant; and V_{max}, maximal enzyme velocity.

in the endoplasmic reticulum of some tissues [16–18]. This form is shown here to be present in the nuclear envelope as well, and a number of its biochemical properties are characterized. This nuclear epoxide hydrolase is distinct from nuclear BPO hydrolase and appears by a number of criteria to be identical to its cytosolic counterpart.

MATERIALS AND METHODS

Tritiated TSO was synthesized and purified as previously described [18]. It was mixed with unlabeled TSO (Aldrich Chemical Co.) that had been purified by column chromatography and recrystallization. The specific activity of the final product used for the assays was 13,500 cpm/nmole. Tritiated BPO was obtained from the National Cancer Institute Chemical Carcinogen Reference Repository, and was diluted before use with unlabeled BPO, obtained from the same source. The specific activity of the product used in the enzyme assays was 950 cpm/nmole. 4-Phenylchalcone oxide (4-PCO) was a gift from Dr. Bruce Hammock, Departments of Entomology and Environmental Toxicology, University of California at Davis. Trichloropropene oxide (TCPO), heparin (Grade I), sucrose (Grade I), *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 2-[*N*-morpholino]ethanesulfonic acid (MES), and tris(hydroxymethyl)aminomethane (TRIS) were purchased from the Sigma Chemical Co., St. Louis, MO. Unlabeled TSO and 4-phenylphenol were purchased from the Aldrich Chemical Co., Milwaukee, WI. Diphenylethanediol was purchased from ICN Pharmaceuticals, Irvine, CA. HPLC-grade methanol was obtained from Fisher Scientific, Pittsburgh, PA. Antiserum to rat liver microsomal BPO hydrolase was obtained as previously described [23]. Goat antiserum to rabbit gamma globulin (GARGG) was obtained from the Calbiochem-Behring Corp., La Jolla, CA.

Nuclei were isolated from the livers of adult male C57B1/6N mice (Harlan Sprague Dawley, Inc., Indianapolis, IN) according to the procedure of Blobel and Potter [24]. This method utilizes a discontinuous sucrose gradient, and, as originally applied to rat liver, results in a preparation of nuclei that is free of contamination by microsomes or other cellular membrane fragments [4, 24]. Preliminary studies showed that the large amounts of nuclear DNA present interfered with the separation by solvent extraction of substrate and product. Therefore, the nuclear envelope fraction was purified by osmotic disruption of the nuclei followed by incubation with heparin to precipitate the nuclear envelope fraction and separate it from the chromatin [25]. Membranes were washed once with 0.25 M sucrose and resuspended. This procedure resulted in an enzyme-containing preparation that was sufficiently free of DNA to permit accurate and reproducible measurement of enzyme activity. Possible cytosolic contamination of the nuclear envelope fraction was assessed using lactate dehydrogenase as marker for cytosolic protein [26].

BPO hydrolase and TSO hydrolase activities were measured by previously described methods; the substrates were removed by solvent extraction, and the remaining diol products were quantitated by liquid scintillation chromatography [12, 16, 18]. Radiolabeled substrates were added in 1–3 μl of acetonitrile. Incubation blanks contained either boiled nuclear membranes or buffer in place of active nuclear envelopes, but always contained the same amount of substrates as the samples to which they were compared. In studies where inhibitors were used, control samples contained 5 μl of methanol, while "inhibited" samples contained appropriate amounts of inhibitor in 5 μl of methanol. I_{50} values were calculated by log-probit analysis, using a published computer program [27]. Protein concentrations were measured by the method of Lowry *et al.* [28].

Epoxide hydrolases were immunoprecipitated from solubilized nuclear envelopes using a protocol previously described for studies using microsomes [23]. Washed nuclear envelopes were solubilized with sodium cholate (final concentration 1%) and incubated with anti-EH1 antiserum. Antigen–antibody complexes were precipitated with a second antibody (GARGG), and epoxide hydrolase activities were measured in the resulting supernatant fraction. BPO hydrolase activity was measured by the standard method described above, but an adapted method employing HPLC separation of substrate and product was required to accurately measure TSO hydrolase activity. Accordingly, 50 μl of the supernatant fraction from the immunoprecipitation was incubated with 30 μl of 200 mM HEPES buffer, pH 6.8, and radiolabeled TSO in 1 μl acetonitrile, resulting in a final substrate concentration of 200 μM. After 60 min of incubation at 37°, the reaction was stopped with 100 μl of ethylacetate. Five nanograms of 4-phenylphenol (1 μg/ml in methanol) was added as an internal standard. The samples were mixed for 30 sec with a vortex mixer and centrifuged to separate the phases. Fifty microliters of the ethyl acetate phase was injected onto the HPLC. The chromatographic separation of product from substrate utilized a Zorbax ODS 4.6 mm × 15 cm column. A gradient elution from 50% methanol/water to 85% methanol/water was run over a period of 20 min at a flow rate of 1 ml/min. Fractions of 1.2 ml were collected directly into plastic vials and processed for liquid scintillation counting. Absorbance of the eluate was monitored at 254 nm, and product recovery was determined by measuring the height of the 4-phenylphenol peak. The rate of product formation was calculated from the number of counts present in the fractions corresponding to the diol multiplied by an appropriate recovery factor. The following elution times were observed: diphenylethane diol, 4.7 min; 4-phenylphenol, 9.5 min; and TSO, 15 min.

RESULTS

Initial experiments designed to measure TSO hydrolase activity in isolated whole mouse liver nuclei were unsuccessful due to interference by DNA with the solvent extraction-based separation of substrate and product. Although product formation was detectable, the assay method was neither sensitive nor reproducible enough to yield interpretable results. However, fractionation of the nuclei and

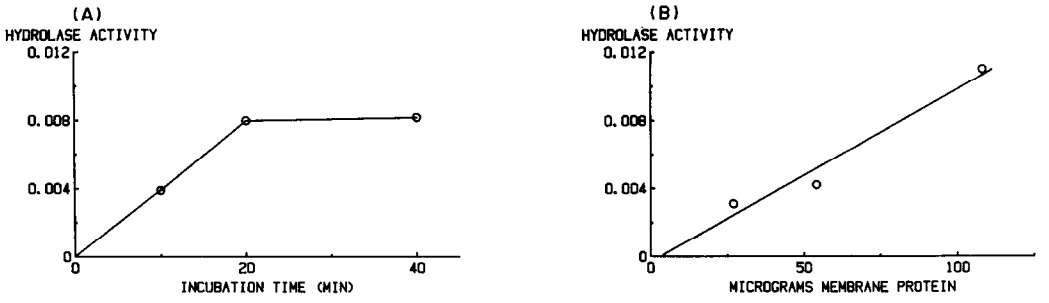

Fig. 1. Dependence of nuclear membrane TSO hydrolase activity on protein concentration and incubation time. (A) Fifty microliters of nuclear membrane preparation, corresponding to 54 μg protein, was incubated for varied times with TSO, and diol production was quantitated. "Hydrolase activity" represents nanomoles of product formed per milliliter. Points are the mean value of triplicate determinations. (B) Varied amounts of membrane protein were incubated with TSO for 15 min, and diol production was quantitated. "Hydrolase activity" is nanomoles of product formed per milliliter. Points represent the mean value of triplicate determinations.

isolation of a purified nuclear envelope fraction provided a preparation containing TSO hydrolase activity that was readily assayable using the solvent extraction method.

Figure 1 shows the results of experiments measuring TSO hydrolase activity in nuclear envelope fractions. Figure 1A shows that initial enzyme velocity was a linear function of incubation time for periods of up to 20 min. Figure 1B shows that initial velocity was also linear with enzyme concentration at protein concentrations of up to 0.1 mg/0.25 ml of incubation mixture. In subsequent studies, an incubation time of 15 min and an amount of membrane preparation corresponding to approximately 50 μg of protein were used. According to the data in Fig. 1, these conditions provide that the rate of product formation observed is an accurate measure of the initial enzyme velocity.

Figure 2 shows the dependence of nuclear TSO hydrolase activity on the pH of the reaction mixture. A pH optimum of approximately 6.4 was seen; this corresponds well to the pH optima previously observed for cytosolic TSO hydrolase activity (6.8–7.0) [16, 22] and microsomal TSO hydrolase activity (7.0) [16]. This value is quite different from the value of 8.5–9.0 observed for microsomal BPO hydrolase [16, 22, 29], and suggests that the enzyme catalyzing the hydration of TSO in nuclear envelope is similar

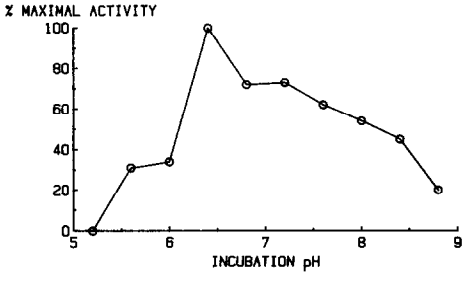

Fig. 2. Dependence of nuclear membrane TSO hydrolase activity on incubation pH. Diol production was quantitated at various incubation pH values. The solutions were buffered from pH 5.2 to 6.4 with MES, from 6.4 to 7.2 with HEPES, and from 7.6 to 8.8 with TRIS. Points represent mean values from triplicate determinations.

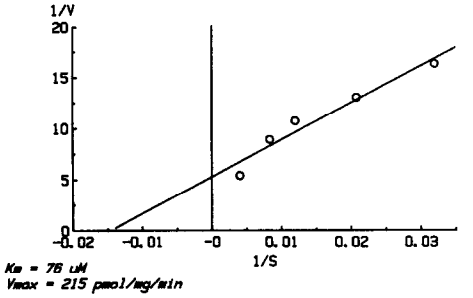

Fig. 3. Kinetics of TSO hydration by nuclear membranes. The dependence of TSO hydrolase activity on substrate concentration was measured using 50 μg of membrane protein incubated for 15 min. Each point represents the mean value of quadruplicate determinations.

to the corresponding microsomal and cytosolic TSO hydrolases, and different from the enzyme catalyzing BPO hydration.

After the initial conditions for optimal measurement of nuclear TSO hydrolase activity had been established, the kinetic constants of the enzyme were determined. Figure 3 shows an inverse reciprocal

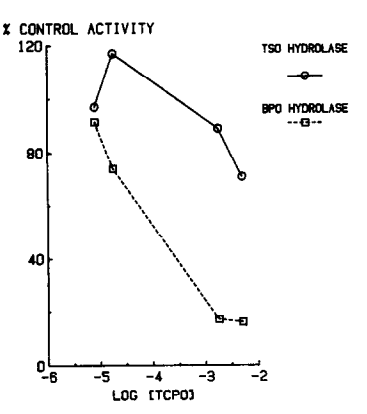

Fig. 4. Inhibition of nuclear membrane epoxide hydrolase activities by TCPO. TSO [—⊖—] and BPO [– -⊟- –] hydrolase activities were measured in the presence of various concentrations of TCPO. Results are expressed as percent of maximal (uninhibited) activity and are the mean value of triplicate determinations.

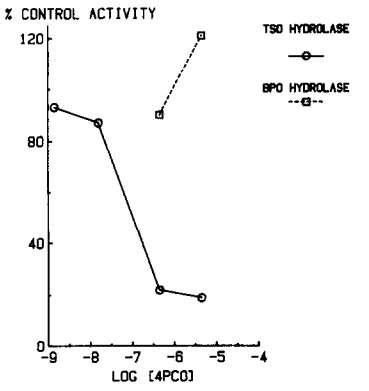

Fig. 5. Inhibition of nuclear membrane expoxide hydrolase activities by 4-PCO. TSO [—⊖—] and BPO [– ⊟ –] hydrolase activities were measured in the presence of various concentrations of 4-PCO. Results are expressed as percent of maximal (uninhibited) activity and are the mean value of triplicate determinations.

plot of velocity versus substrate concentration. This figure is included merely as graphic representation of the data; kinetic parameters were calculated directly from the data itself [30]. A V_{max} of 215 pmoles product per min per mg membrane protein, and a K_m of 76 μM were calculated. Nuclear envelopes, therefore, contain fairly high amounts of this enzyme activity; the V_{max} observed is about three times that observed in mouse liver microsomes [16], but less than that seen in the cytosol [16]. On the other hand, the K_m for nuclear TSO hydrolase is about seven times that of either microsomal or cytosolic TSO hydrolase [16], indicating that the nuclear enzyme has relatively low affinity for the substrate.

To assess both the degree of catalytic similarity between nuclear TSO hydrolase and its microsomal and cytosolic counterparts and the differences between nuclear BPO hydrolase and nuclear TSO hydrolase, the response of the enzyme to *in vitro* inhibition by two compounds was determined. TCPO is a potent inhibitor of microsomal BPO hydrolase activity [18, 31]. As Fig. 4 shows, it also inhibited nuclear BPO hydrolase activity, with an I_{50} concentration of 2×10^{-4} M. However, nuclear TSO hydrolase activity was not inhibited at all by this compound at concentrations below 10^{-3} M. The difference in response of these two activities to inhibition by TCPO suggests that they are catalyzed by different enzymes. The potent inhibitor of microsomal and cytosolic TSO hydrolase activity, 4-phenylchalcone oxide (4-PCO) [18, 32], was also used in attempts to inhibit nuclear hydrolase activities. As Fig. 5 shows, 4-PCO readily inhibited nuclear TSO hydrolase activity at submicromolar concentrations; the I_{50} was calculated as 1.7×10^{-7} M. However, even at concentrations 100-fold higher than this, no inhibition of nuclear BPO hydrolase activity was observed. The sensitivity of nuclear TSO hydrolase to inhibition by 4-PCO again suggests that this enzyme is catalytically similar to microsomal and cytosolic TSO hydrolases, but different from nuclear or microsomal BPO hydrolase.

The apparent kinetic differences between nuclear TSO hydrolase activity and nuclear BPO hydrolase activity may be attributable to the fact that they represent one enzyme whose catalytic properties vary greatly depending on the substrate or, alternatively, that they represent two distinct enzyme proteins. If the latter were the case, then we would expect structural differences between the two enzymes to be reflected in their differing affinities for epoxide hydrolase-specific antibodies. To test this hypothesis, immunotitration experiments were designed. Solubilized nuclear envelopes were incubated with antiserum to rat liver microsomal EH1 (BPO hydrolase). After precipitation of antibody–enzyme complexes, non-immunoprecipitable enzyme activity was measured. However, the solvent extraction method used to measure TSO hydrolase activity in unadulterated membrane fractions did not prove satisfactory for measurement of activity after immunoprecipitation, due primarily to the interference with extraction of substrate by the large amounts of protein present. Therefore, a modification of the assay was devised whereby both substrate and product were extracted from the incubation mixture and separated by HPLC, allowing for a great improvement in sensitivity and reproducibility of product quantitation. Because the activities measured using this method were low, they were a linear function of enzyme concentration even at 60 min of incubation time (data not shown). BPO hydrolase activity could be readily measured by the normal solvent extraction method, and HPLC separation was not required. Figure 6 shows the titration of nuclear envelope epoxide hydrolase activities with antiserum to microsomal EH1. Nuclear BPO hydrolase activity was precipitated by the antibody, demonstrating that there is sufficient structural similarity between nuclear and microsomal BPO hydrolases to permit cross-reactivity with the antibody. On the other hand, nuclear TSO hydrolase activity was not complexed by the antibody. This suggests that nuclear TSO hydrolase has little or no structural

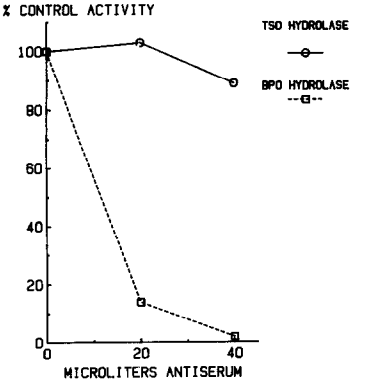

Fig. 6. Immunotitration of nuclear membrane epoxide hydrolase activities by antiserum to rat liver microsomal BPO hydrolase. Solubilized membrane preparations were titrated with antiserum as described in Materials and Methods. TSO [—⊖—] and BPO [– ⊟ –] hydrolase activities of non-immunoprecipitated proteins were measured and are expressed here as percent of control activity, where control activity is that activity present after incubation with nonimmune rabbit serum. Points represent the mean of values from triplicate determinations.

homology with microsomal BPO hydrolase or, by extension, with nuclear BPO hydrolase. Nuclear TSO and BPO hydrolases appear, therefore, to be two catalytically and structurally distinct enzymes.

DISCUSSION

The concept once held, that xenobiotic-metabolizing enzymes are strictly compartmentalized into either microsomal or cytosolic cell fractions, has been modified by the realization that a number of these enzymes have corresponding forms that are microsomal, nuclear and/or cytosolic. Cytochrome P-450 isozymes, thought at one time to be strictly located in the endoplasmic reticulum, are also found in the nuclear envelope [6, 20, 33]. Cytosolic, mitochondrial, and microsomal forms of glutathione-*S*-transferase exist [34, 35]. The epoxide hydrolase that hydrates BPO and other arene oxides, designated here as EH1, is located primarily in the endoplasmic reticulum [1–3]. Subsequent to the original demonstration of its location in microsomes, however, EH1 has also been found in the nuclear envelope [5, 6, 21], and in some species, including man, in significant amounts in the cytosol [7–9, 36]. A second epoxide hydrolase, originally shown by Ota and Hammock to participate in the mammalian metabolism of certain substituted alkene-derived epoxides [22], is located primarily in the cytosol of most species. This enzyme is designated here as EH2. It was subsequently demonstrated that epoxide hydrolases indistinguishable from this cytosolic enzyme are located in the lumen of the mitochondrion [13, 14], and in the endoplasmic reticulum of a number of species, including man [7, 16, 18]. In this study, we provide evidence that an epoxide hydrolase with properties very similar to those of EH2 is found in the nuclear envelope. We also demonstrated that this enzyme was distinct from the nuclear enzyme corresponding to EH1.

Initial attempts to quantitate TSO hydrolase activity in preparations of whole nuclei were frustrated by interference by DNA with the extraction of the radiolabeled substrate out of the incubation mixture. Therefore, a chromatin-free nuclear envelope fraction was isolated and used as an enzyme source for the *in vitro* assays. The pH optimum for this nuclear enzyme activity, pH 6.4, was established by the experiment illustrated in Fig. 2. These results provide evidence that the enzyme responsible for nuclear TSO hydrolase activity is catalytically similar to both cytosolic and microsomal TSO hydrolases, which both have pH optima in the 6.5–7.5 range [16, 22]. Furthermore, these data suggest that nuclear TSO hydrolase activity is not catalyzed by EH1, which is known to have a pH optimum of 8.5–9.0 for several substrates [2, 29].

Conclusive evidence of the involvement of a nuclear membrane-bound enzyme in TSO hydration is offered in Fig. 3. The kinetics conform to the classic Michaelis–Menten model, as the inverse reciprocal plot indicates [37]. The V_{max} of the nuclear enzyme, 215 pmoles per min per mg protein, is lower than that previously calculated for cytosolic EH2 (1.5 nmoles per min per mg [16], or 6.9 nmoles per min per mg [32]) but is higher than that of the corresponding microsomal enzyme (75 pmoles per min per mg) [16]. The concentration of enzyme in the nuclear envelope appears, therefore, to be greater than that in the endoplasmic reticulum, but less than that found in the cytosol. The apparent K_m of the nuclear enzyme, 76 μM, was greater than that previously calculated for either cytosolic or microsomal TSO hydrolase activity (11 and 10.3 μM respectively) [16]. The affinity of the nuclear enzyme for TSO is apparently not as great as either microsomal or cytosolic EH2. This may be due to structural differences in the enzymes themselves near the active site, which are expressed as differences in kinetic parameters. Alternatively, differences in enzyme conformation caused by differences in the environments surrounding the enzymes in the nuclear or microsomal membrane, or in the cytosol, may cause the apparent differences in substrate affinity. These results should be interpreted cautiously, as apparent K_m values determined using membrane fragments *in vitro* can depend on the concentrations of membrane protein and lipid present [38].

That the active site of nuclear TSO hydrolase is very similar to those of cytosolic and microsomal EH2 is suggested by the response of this enzyme to two enzyme inhibitors *in vitro*. The halogenated epoxide TCPO inhibits both nuclear and microsomal EH1 at micromolar concentrations [4, 18, 31]. It also inhibits cytosolic and microsomal EH2, but only at much higher (millimolar) concentrations [18, 32]. The effects of TCPO on nuclear epoxide hydrolase activities are shown in Fig. 4. Nuclear BPO hydrolase activity was inhibited by TCPO at micromolar inhibitor concentrations, as expected. Nuclear TSO hydrolase activity was not inhibited at TCPO concentrations below 10^{-4} M. This provides further evidence that nuclear TSO hydrolase activity was due to an enzyme different than nuclear EH1, and that the catalytic properties of nuclear TSO hydrolase were very similar to those of microsomal and cytosolic EH2.

The effects of a second inhibitor, 4-PCO, on nuclear epoxide hydrolase activities were also examined. This compound is highly selective for EH2 in cytosol and microsomes; it inhibits TSO hydrolase activities in these subcellular fractions at nanomolar concentrations, but has no discernible effect on EH1-catalyzed activity even at 10^{-5} M concentrations [18, 32]. Use in this study of higher inhibitor concentrations was precluded by solubility limitations. 4-PCO inhibited nuclear TSO hydrolase activity at very low concentrations (Fig. 5); the I_{50} concentration was calculated as 1.67×10^{-7} M. This value is higher than the I_{50} concentrations previously calculated for cytosolic and microsomal TSO hydrolase activities (64 and 68 nM respectively) [18]. Even at relatively high concentrations (10^{-6} and 10^{-5} M), no inhibition of BPO hydrolase activity was seen. These data further support the conclusion that nuclear BPO and TSO hydrolase activities are catalyzed by different enzymes. Nuclear TSO hydrolase activity is similar to microsomal and cytosolic EH2 in that all three are inhibited by 4-PCO. The higher I_{50} concentration observed for the nuclear enzyme indicates a somewhat lower affinity of the enzyme for the inhibitor, just as its higher K_m value indicates

a lower affinity for TSO. Again, this may indicate a major structural difference at the active site, or, more probably, the effects of different enzyme environments. At any rate, the similarities in sensitivity to the two inhibitors of nuclear TSO hydrolase with those of cytosolic and microsomal EH2 indicate that these three forms are catalytically very similar. Lactate dehydrogenase activity, a well characterized marker for cytosolic protein, was not demonstrable in the nuclear envelope fraction at a level of detection that would theoretically show a 0.02% contamination by cytosol (data not shown). We conclude, therefore, that the nuclear enzyme, while very similar to its cytosolic counterpart, is not an artifact of cytosolic contamination.

Immunoprecipitation experiments offer conclusive proof that nuclear TSO hydrolase activity is catalyzed by an enzyme that is structurally distinct from nuclear EH1. Because of the total absence of reactivity of TSO hydrolase with anti-EH1 antiserum, we conclude that major structural differences between the two enzymes exist. On the basis of the absence of cross-reactivity of these two nuclear envelope epoxide hydrolase activities with the antiserum, we conclude that the catalytic differences demonstrated by their different pH optima and their different responses to *in vivo* inhibitors reflect catalysis by structurally distinct enzymes.

In conclusion, we have demonstrated the existence of an epoxide hydrolase in the nuclear envelope that is by the criteria of its catalytic and structural properties distinct from the previously described nuclear arene oxide hydrolase (EH1). This nuclear epoxide hydrolase has many properties in common with the TSO hydrolases known to be present in microsomes and cytosol. The structural relationship between this nuclear TSO hydrolase and microsomal and cytosolic EH2 remains to be elucidated. Because this enzyme is located in the nucleus, in close proximity to the target site for many toxic epoxide intermediates, and because this hydrolase can detoxify epoxides not metabolized by the other nuclear epoxide hydrolase, the enzyme described here may endow the cell with an additional means of protection against an unpredictable chemical environment.

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