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SHORT COMMUNICATION

SOLUBLE MAMMALIAN EPOXIDE HYDRATASE: ACTION ON JUVENILE HORMONE AND OTHER TERPENOID EPOXIDES*

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Abstract—1. A soluble epoxide hydratase from mouse liver or kidney converts juvenile hormone and two epoxide containing juvenoids to their respective diols faster than the microsomal epoxide hydratases.

2. The soluble enzyme hydrates the two trisubstituted epoxides much faster than the tetrasubstituted epoxide.

3. Analysis by gel filtration indicates an approximate mol. wt of 150,000 for the liver enzyme while isoelectric focusing indicates a pI of 4·9.

INTRODUCTION

ALTHOUGH epoxide hydratases (hydrases) (E.C. 4.2.1.63) are known to mediate the conversion of the epoxide (oxirane) ring to a 1,2-diol, the function of epoxide hydration is not completely understood. However, the importance of epoxide hydratase (EH) in the degradation of potentially toxic and carcinogenic xenobiotics is well known (see Oesch, 1972). It has been shown that EH appears to be critical for the biodegradation of juvenile hormone (JH) in some insects (Slade & Zibitt, 1972) as well as for the breakdown of JH mimics (juvenoids) (Gill *et al.*, 1972; Hammock *et al.*, 1974*a*), some of which are regarded as potential agents for insect control. Since some juvenoids may soon become constituents of our environment, it was of interest to study mammalian EH action on JH and some juvenoids.

With the substrates previously examined, almost all mammalian EH activity is membrane-bound with most of the activity in the microsomal fraction (see Oesch, 1972). However, in the course of studying juvenoid metabolism in mammalian tissues, it was noted that the EH activity of liver and kidney homogenates was in the soluble fraction when one juvenoid (compound A, Table 1) was used as substrate (Gill *et al.*, 1972, 1974). In contrast, all studies using juvenile hormone (compound B, Fig. 1), juvenoids, and cylcodiene epoxides so far indicate that insect EH activity is membrane bound (Brooks *et al.*, 1970; Hammock *et al.*, 1974*a*; M. Slade, personal communication). This report describes the partial characterization of the soluble mammalian EH and its action on JH and two juvenoids.

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MATERIALS AND METHODS

Mice (male Swiss Webster, 18–22 g) were killed by cervical dislocation and the liver and kidney tissues removed immediately and washed. Cold phosphate buffer (pH 7·4, 0·2 M) was added (20% w/v) followed by homogenization (Sorvall omnimixer). Soluble and microsomal fractions were obtained by initial centrifugation (12,000 ***g***; 15 min), glass wool filtration and ultracentrifugation (105,000 ***g***; 60 min). For time dependent studies the soluble fraction was diluted with buffer to a concentration of 4 mg/ml-protein and microsomes were diluted to an equivalent volume giving 0·4 mg/ml protein (Lowry *et al.*, 1951) while kinetic studies were conducted with partially purified fractions (0·1 mg/ml) from a Sephadex G-150 column.

The structures of the three radiolabeled substrates used are shown in Table 1. Compound A was labeled with tritium (Kamimura *et al.*, 1972) with a specific activity of about 600 mCi/m-mole. Tritium labeled compound B (about 14 Ci/m-mole) was purchased from New England Nuclear Corp. (Boston, Mass.) while the cold compound was provided by Zoecon Corporation (Palo Alto, Calif.). Both ^{14}C-labeled (about 11 mCi/m-mole) and cold samples of compound C were provided by Hoffman–LaRoche & Co. (Basle, Switzerland). The diol standards were prepared by acid cleavage (Gill *et al.*, 1972) and their structure verified by proton magnetic resonance.

Enzyme incubations were at 37°C with gentle shaking, in vials coated with carbowax (polyethylene glycol mol. wt *ca.* 20,000) (Hawk *et al.*, 1972) to prevent adherence of the lipophilic substrates to the glass. Substrates were added in ethanol (< 1% final vol) and the reaction was terminated by the addition of ammonium sulfate and 3 vols of ethyl

Table 1. Epoxide hydratase activity of mouse liver and kidney microsomes and soluble fractions[a]

Substrate	Microsome activity		Soluble activity		
	Liver	Kidney	Liver	Kidney	Liver (partially purified)
A. Stauffer R-20458	0.4 (0.16)	<0.1	0.4 (1.6)	0.6 (2.4)	7.6 ± 0.4
B. C_{18} Juvenile hormone	<0.1	<0.1	0.6 (2.4)	1.1 (4.4)	6.4 ± 0.3
C. Hoffman La Roche Ro-8-4314	--[b]	--[b]	--[b]	--[b]	2.0 ± 0.1

a) Activity is expressed as nmoles of substrate converted (hydrated)/min/mg protein. Numbers in parentheses denote activity expressed in tissue equivalents. The reaction mixtures contained 0.1 μmole substrate and 0.4 mg protein (microsomes) or 4 mg protein (soluble fraction). The reaction mixture for the partially purified liver soluble enzyme contained 0.5 μmole substrate and 0.1 mg protein.

b) Extremely low activity was observed with some ester cleavage.

ether (containing 0·01% pyridine to stabilize epoxides; J. J. Menn, personal communication) for compound A or ethyl acetate (containing 0·01% pyridine) for compounds B and C (Fig. 1). Greater than 95% of the radioactivity was recovered by this method. The organic phase was removed, dried, concentrated and subjected to silica gel thin-layer chromatography (TLC) along with appropriate standards. The TLC solvent system routinely used for compound A was benzene:*n*-propanol (10:1) while that for compound B and C was hexane:ethyl acetate (3:1). One and two dimensional plates were run in an attempt to discern all metabolites. The bands corresponding to authentic diol and epoxide were scraped and analyzed by liquid scintillation spectrometry. All values were corrected for non-enzymatic diol formation. Qualitative analyses of TLC plates were conducted by TLC scanning (Packard Instruments, Model 7201) and autoradiography (Randerath, 1970). The radiolabeled metabolites of the substrates (A, B, C in Table 1) were identified as diols by co-chromatography of the metabolites and their acetate (except for compound C) and *n*-butyl boronic acid derivatives with authentic standards prepared by previously described methods (Hammock *et al.*, 1974*b*) and identified spectrophotometrically.

RESULTS AND DISCUSSION

Initial experiments revealed a linear increase in the amount of substrate degraded to the diol as a function of 105,000 *g* supernatant protein in the range of 0·2 mg to 4·5 mg protein. Beyond this protein concentration, EH activity decreased. The hydration of the epoxide to the diol is time-dependent with more activity exhibited by the liver or kidney soluble fractions than by their respective microsomal fractions when expressed as tissue equivalents (Table 1). Centrifugation at 150,000 *g* for 2 hr yields the same EH activity in the microsomal and soluble fraction as centrifugation at 105,000 *g* for 1 hr, suggesting the presence of a soluble enzyme. These data, the observation that enzyme activity from the soluble fraction is completely precipitated with 90% ammonium sulfate but resolubilized upon removal of the salt and the loss of activity upon heat denaturation or pronase treatment, all support the supposition that the reactions studied are enzyme mediated.

It is of interest that we detected very little ester cleavage of compounds B or C with the soluble fraction and essentially none with the partially purified fraction from gel filtration (see below) although there are rather high levels of esterases in mammalian liver acting on other substrates. Since one route of JH degradation in insects proceeds via soluble carboxylesterase mediated ester hydrolysis (Slade & Zibitt, 1972), this finding indirectly supports the premise that the insect esterases responsible for degrading JH are enzymes specific for JH.

Although precise values for the V_m and K_m for the three compounds were not obtained, at every substrate level (0·2–0·01 μmole/ml) and incubation time (15–120 min) examined with the partially purified enzyme, compound A was hydrated faster than B which was hydrated much more rapidly than C. The failure of EH to rapidly metabolize the tetra-substi-

tuted epoxide (compound C) may indicate that this compound does not fit well at the active site or that a nucleophilic attack on a tri-substituted carbon is important in the activation or hydration of the epoxide moiety.

Further characteristics of the soluble EH were obtained by gel filtration (Sephadex G-150 and G-200), ion exchange chromatography (Sephadex CM 50 and DEAE 50) and isoelectric focusing (LKB 8100 ampholine electrofocusing column). The pI of the soluble EH is 4·9 and a 2-fold purification was attained by ion exchange chromatography. Gel filtration yielded a 20 to 30-fold purification with high recovery of the enzyme activity from the column. The estimated mol. wt of the soluble EH is 150,000 (assuming a globular structure) and is based on chromatography through a 90 cm column of Sephadex G-150 or G-200 superfine along with known markers. The soluble EH therefore appears to be almost three times the mol. wt of experimentally solubilized guinea-pig liver microsomal EH (Oesch, 1972). Although the results strongly suggest the presence of a soluble EH in the mouse distinct from the microsomal enzyme, the possibility that solubilization occurred during the extraction procedure cannot be completely excluded. However, our regimen failed to cause solubilization of insect fat body or gut EH. We believe that the major reason for the failure of other workers to detect a soluble EH in mammalian systems is a consequence of the substrates routinely utilized (Oesch *et al.*, 1971; Oesch, 1972). Although more substrates must be examined, perhaps the reason that the soluble EH is more active than the microsomal EH in hydrating compounds A and B and JH is that the soluble enzyme shows some specificity for lipophilic epoxides and therefore may also be involved in the hydrolysis of epoxidized fatty acids, steroids or their precursors, and xenobiotics such as the epoxide containing terpenes present in some citrus oils (Stanley & Jurd, 1971).

In addition to the potential importance of a soluble EH in the detoxification of carcinogenic compounds, degradation of juvenoids used for pest control, etc., the utilization of this enzyme should facilitate studies on the mechanism of action of epoxide hydratases as well as provide a useful microcatalyst for the conversion of epoxides to diols.

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