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The Reaction of Arachidonic Acid Epoxides (Epoxyeicosatrienoic Acids) with a Cytosolic Epoxide Hydrolase[1]

N. CHACOS, J. CAPDEVILA, J. R. FALCK,* S. MANNA,* C. MARTIN-WIXTROM, S. S. GILL,† B. D. HAMMOCK,‡ AND R. W. ESTABROOK

*Department of Biochemistry and *Department of Molecular Genetics, Southwestern Medical School, University of Texas Health Science Center, Dallas, Texas 75235; and †Department of Entomology, University of California, Riverside, California, 92521; and ‡Department of Entomology, University of California at Davis, Davis, California 95616*

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Epoxyeicosatrienoic acids, formed during the cytochrome *P*-450-catalyzed oxidation of arachidonic acid, react with a liver cytosolic epoxide hydrolase to form vicinal diols of eicosatrienoic acid. The role of this cytosolic enzyme, rather than a microsomal bound type, explains previous results illustrating the ability to accumulate epoxides during the *in vitro* aerobic steady state of oxidative metabolism of arachidonic acid by liver microsomes. The inability of the 5,6-epoxyeicosatrienoic acid to serve as a suitable substrate for this enzyme is discussed in light of recent studies concerning possible unique physiological functions for this metabolite.

The oxidation of arachidonic acid to a variety of metabolites by an NADPH and cytochrome *P*-450-dependent reaction of liver microsomes has been reported recently (1). Approximately 40 to 50% of the metabolites formed during the steady state oxidation of arachidonic acid by rat liver microsomal cytochrome *P*-450 were epoxyeicosatrienoic acids (EETs)[2] sufficiently stable to be isolated by HPLC and characterized by mass spectral analysis (2). Oliw *et al.* (3, 4) have recently reported a role for a rabbit liver microsomal epoxide hydrolase in the hydration of these arachidonic acid epoxides to their respective vicinal dihydroxy derivatives, thereby explaining their observation that during studies with rabbit liver microsomes significant amounts of dihydroxy derivatives were formed when arachidonic acid was metabolized by cytochrome *P*-450. The purpose of this paper is to attempt to resolve this apparent discrepancy and to evaluate the role of a cytosolic epoxide hydrolase, in contrast to a comparable microsomal-bound enzyme, in the hydration of arachidonic acid epoxides to their respective vic-dihydroxy derivatives.

The isolation and purification of an epoxide hydrolase (EC 3.3.2.3) associated with the microsomal fraction of liver has been well established (5–7). This enzyme is reactive toward a number of arene oxides formed during the biotransformation of aromatic compounds by the liver microsomal cytochrome *P*-450 system. The pres-

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[2] In analogy to the acronym utilized for the lipoxygenase products (HETE) we would like to introduce the term EET to describe the products of the epoxygenase pathway of metabolism of arachidonic acid, i.e., the epoxyeicosatrienoic acids. The position of the oxido group is described by the carbon atoms to which oxygen is bonded, e.g., 5,6-epoxyeicosatrienoic acid is abbreviated as 5,6-EET. Correspondingly, the vic-diols resulting from hydration of the EETs are designated by the acronym DHET. Thus the 5,6-dihydroxyeicosatrienoic acid is abbreviated as 5,6-DHET.

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ence of additional epoxide hydrolases, in cells such as hepatocytes, has been a point of dispute although clear evidence for the presence of a cytosolic enzyme has been presented (8).

The role of oxidation products of arachidonic acid, such as prostaglandins, thromboxanes, and leukotrienes, in mediating a number of reactions of both physiologic and pharmacologic importance is well documented. Recent studies have implicated the involvement of some of these novel epoxides, formed during the cytochrome *P*-450 oxidation of arachidonic acid, in the *in vitro* release of some peptide hormones (9, 10). A knowledge of the enzymatic reactions in which these arachidonic acid epoxides participate as substrates therefore was considered of importance. The present report demonstrates that the cytosolic epoxide hydrolase of liver, in contrast to a microsomal-bound enzyme, rapidly hydrates the 8,9-, 11,12-, and 14,15-EETs with only a slow rate of hydrolysis of the 5,6-EET.

MATERIALS AND METHODS

Microsomes were prepared from the livers of male Sprague-Dawley rats (150-200 g) which had been treated by daily intraperitoneal injections of phenobarbital (80 mg/kg body weight) for 5 days as described (11). The microsomal fraction was separated by differential centrifugation from 20% homogenates (w/v) prepared using 0.25 M sucrose. The supernate from the initial high spin centrifugation at 105,000*g* was used as the source of the cytosolic fraction.

The products of the microsomal cytochrome *P*-450-dependent oxidation of arachidonic acid were isolated from incubation mixtures containing 10 mM $MgCl_2$, 150 mM KCl, 50 mM Tris-Cl (pH 7.5), 8 mM sodium isocitrate, 0.25 I.U./ml of isocitrate dehydrogenase, 0.1 mM [1-^{14}C]arachidonic acid (0.6 mCi/mmol), 0.5 mM NADPH, and liver microsomes (0.5 mg/ml). The reaction mixtures were incubated at 25°C with constant mixing for the designated periods of time. The reaction products were extracted with ethyl acetate and separated by HPLC on a C_{18} μBondapak column as described previously (1, 2).

The rat liver cytochrome *P*-450 containing monooxygenase system was reconstituted by combining in a 1:1:1 molar ratio in dilauroylglycero-3-phosphocholine the following purified components: cytochrome *P*-450, NADPH-cytochrome *P*-450 reductase and cytochrome b_5. The dilauroylglycero-3-phosphocholine were dispersed by sonication and added to give a concentration of 100 μg/nmol of cytochrome *P*-450. The incubation conditions as well as product extraction and resolution was done as in (12). Cytochrome b_5 was included in the reaction system since previous studies (12) have shown that this hemeprotein causes a two- to three-fold enhancement in the rate of arachidonic acid metabolism. The effect of partially purified epoxide hydrolases on arachidonic acid metabolism by this reconstituted system was studied by adding the epoxide hydrolase to the reaction mixture prior to the initiation of the reaction by the addition of NADPH.

The effect of either the cytosolic fraction or a partially purified epoxide hydrolase on the liver microsomal metabolism of arachidonic acid was analyzed by adding them to the incubation mixture prior to initiation of the reaction by the addition of NADPH.

Cytochrome *P*-450 was purified to a specific content of 17.5 nmol cytochrome/mg protein, from microsomal fractions isolated from the livers of phenobarbital-treated male rats, as described by Imai and Sato (13). NADPH-cytochrome *P*-450 reductase and cytochrome b_5 were purified to homogeneity from pig liver microsomes as described by Yasukochi and Masters (14) and Strittmatter *et al.* (15), respectively.

Cytosolic epoxide hydrolase was partially purified from mouse liver as follows: crude mouse liver cytosol (160 ml, 12% w/v homogenate) in 0.25 M sucrose containing 20 mM Tris-HCl, pH 7.6, was applied to a DEAE-cellulose (DE-52) column (2.5 × 20 cm), which was eluted first with 20 mM Tris-HCl and then with a gradient of 0 to 0.2 M NaCl in 20 mM Tris-HCl. The cytosolic epoxide hydrolase which elutes at 70-90 mM NaCl was collected (160 ml) and concentrated by ultrafiltration. The concentrate, adjusted with Tris-HCl and NaCl to give a solution (25 ml) 0.35 M NaCl in 100 mM Tris-HCl, pH 7.6, was then applied to a Phenyl-Sepharose column (1.1 × 20 cm), which was eluted first with 0.35 M NaCl containing 100 mM Tris-HCl, pH 7.6, and then with a gradient of 100 mM Tris-HCl, pH 7.6, to 5 mM Tris-HCl, 2% ethylene glycol, and 0.1 mM dithiothreitol (DTT). Fractions eluting between 0.2 and 1% ethylene glycol were combined (123 ml) and concentrated by ultrafiltration. Table I summarizes the increase in specific activity obtained during the partial purification of this enzyme. This partially purified enzyme preparation has been shown to be free of glutathione and glutathione *S*-transferase activity.

The 5,6-, 8,9-, 11,12-, and 14,15-epoxides of arachidonic acid were synthesized from [1-^{14}C]arachidonic acid as described by Corey *et al.* (17, 18) and Falck and Manna (19).

RESULTS

The effect of liver cytosol on the profile of metabolites formed during the microsomal metabolism of arachidonic acid. In-

TABLE I

PARTIAL PURIFICATION OF A MOUSE LIVER CYTOSOLIC EPOXIDE HYDROLASE

Fraction	Volume (ml)	mg protein	Specific activity	Total activity	Recovery	Purification
Cytosol	160	483	7.6	3670	100	1
DE-52	160	139	20.1	2790	76	2.6
Phenyl-Sepharose	123	10.5	132	1390	38	17.4

Note. Activity was assayed with *trans*-stilbene oxide as described (16). Units of specific activity are expressed as nmol/min/mg protein while total activity is nmol/min. The Phenyl-Sepharose fraction had an activity of 7.6 nmol/min/mg protein for the hydration of *cis*-stilbene oxide and was assayed at pH 9.0. Details of the fractionation procedures are described under Materials and Methods.

cubation of rat liver microsomes with NADPH and arachidonic acid results in the formation of a variety of metabolites, as illustrated by the HPLC profile shown in Fig. 1 (Curve A). Many of these metabolites have been isolated and identified (2, 20). These include various monohydroxy acids (HETEs) (retention times of 18 to 22 min) and epoxy acids (EETs) (retention times of 23 to 25 min). Table II summarizes

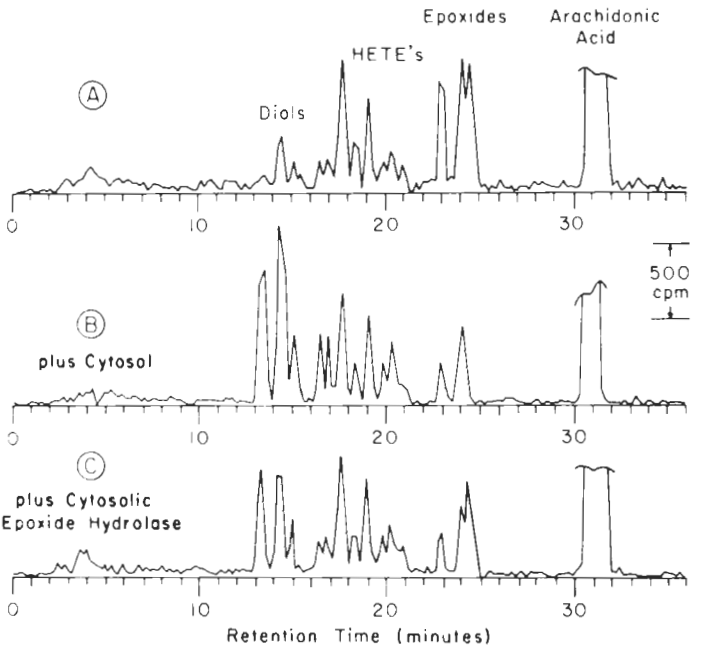

FIG. 1. The effect of liver supernate and partially purified cytosolic epoxide hydrolase on the profile of metabolites formed during the liver microsomal *P*-450-dependent oxidation of arachidonic acid. Rat liver microsomes (0.5 mg protein/ml) from phenobarbital-treated animals were incubated for 5 min at 25°C with 100 μM [1-^{14}C]arachidonic acid, 0.3 mM NADPH, and an NADPH-generating system in a buffer mixture containing 50 mM Tris–Cl, 10 mM $MgCl_2$, and 150 mM KCl. The reaction was terminated by addition of acidified ethyl acetate and extracted and analyzed by HPLC as described under Materials and Methods. The HPLC profile of radioactive metabolites in the absence of further additions is shown in Curve A. The results of experiments where the incubation mixture was supplemented with either 4.4 mg of the 105,000*g* supernatant of rat liver of 10 μg of a partially purified mouse liver cytosolic epoxide hydrolase are shown in Curves B and C, respectively.

TABLE II

COMPARATIVE RETENTION TIMES OF ARACHIDONIC ACID METABOLITES

Compound[a]	Retention time (min)
14,15-DHET	13.2
11,12-DHET	14.3
8,9-DHET	15.0
5,6-DHET	15.3
15-HETE	18.7
11-HETE	19.5
12-HETE	20.0
8-HETE	20.6
9-HETE	20.6
5-HETE	20.7
14,15-EET	22.5
11,12-EET	23.2
8,9-EET	23.6
5,6-EET	23.9
Arachidonic acid	31

[a] The conditions for resolution of the various metabolites by HPLC analysis on a C_{18}-μBondapak column using an acidified acetonitrile–water gradient are those described under Materials and Methods and in Ref. (1).

the retention times of those metabolites identified to date using the conditions of HPLC analysis described under Materials and Methods. Indeed, under the conditions of the experiments described in Fig. 1 (Curve A), a significant amount of the products formed during the time-dependent oxidation of arachidonic acid by liver microsomal cytochrome *P*-450 are epoxides which remain stable during the incubation and subsequent extraction and HPLC analysis. All four possible monoepoxyacid derivatives capable of formation by attack by an "active oxygen" at the double bonds of arachidonic acid, have been isolated, identified, and their structural assignments confirmed by chemical synthesis. At difference with the arachidonic acid epoxides generated from intermediate hydroperoxides, the products formed during the microsomal cytochrome *P*-450-directed oxidation of arachidonic acid do not contain a conjugated triene functionality in their molecular structure and are therefore chemically different from those of the leukotriene type of metabolite.

In experiments in which the incubation mixture was supplemented with an aliquot of the cytosolic fraction of rat liver, the metabolite profile changed dramatically. A large decrease in the relative content of epoxides formed, concomitant with an increase in more polar metabolites (retention times 14 to 16 min) was evident (Fig. 1, Curve B). These metabolites coelute with the synthetic vic-dihydroxyeicosatrienoic acids (DHETs) (Table II) and correspond to the vic-dihydroxy derivatives formed by hydration of the corresponding epoxides. The amount of new metabolites generated was essentially stoichiometric with the amount of epoxides normally formed in the absence of added cytosol. This type of experiment indicates that the cytosol of liver contains an enzyme (or factor) responsible for the hydration of these epoxides, and in addition suggests a role for a cytosolic epoxide hydrolase in this reaction, rather than that of the comparable microsomal-bound enzyme.

A third experiment was carried out in which the microsomal incubation mixture was fortified with a small amount of a partially purified mouse liver cytosolic epoxide hydrolase. Under conditions of incubation, extraction, and analysis, comparable to those described for the experiments illustrated in Fig. 1 (Curves A and B), the steady level of epoxides was over 50% reduced with a concomitant increase in the appearance of more polar dihydroxy metabolites (Fig. 1, Curve C). A balance of the amount of epoxides or diols formed under the various experimental conditions described in Fig. 1, is shown by the data presented in Table III. The overall rate of arachidonic acid oxidation was not significantly altered by the presence of either liver cytosol or the partially purified cytosolic epoxide hydrolase, indicating the absence of an inhibitory effect of the epoxides on the function of microsomal cytochrome *P*-450 under these experimental conditions, i.e., low protein concentration of liver microsomes and relatively short incubation times.

Studies with a reconstituted system. Since

TABLE III

THE EFFECT OF CYTOSOL OR EPOXIDE HYDROLASE ON THE BALANCE OF PRODUCTS FORMED DURING THE METABOLISM OF ARACHIDONIC ACID

Experimental conditions	Rate of arachidonic acid metabolism[a]	Rate of epoxide formation[a]	Rate of diol formation[a]
Control	19.6	5.2	1.5
Plus cytosol	18.3	1.9	5.7
Plus cytosolic epoxide hydrolase	18.5	2.8	4.1

[a] Rates are expressed as nmol/5 min/mg microsomal protein. The amount of epoxide metabolites was determined from the sum of the radioactive products formed eluting between 22 and 25.5 min. The amount of diol metabolites formed was determined from the radioactivity present eluting between 12 and 15.5 min. Experimental conditions are those described in the legend for Fig. 1.

liver microsomes consist of a heterogeneous population of membranes, which contain a variety of enzymes and lipids, a series of studies were carried out using a reconstituted electron transport system consisting of purified liver cytochrome *P*-450, NADPH-cytochrome *P*-450 reductase, cytochrome b_5, and dilauroylglycero-3-phosphocholine. Previous studies (12) had shown that this system was capable of oxidizing arachidonic acid principally to epoxides in a reaction that was significantly stimulated by the presence of cytochrome b_5. As shown in Fig. 2, inclusion of partially purified cytosolic epoxide hydrolase to the reconstituted enzyme system nearly completely prevented the accumulation, during the steady state, of measurable amounts of epoxides (retention times of 23 to 25 min) and resulted in an increase of the more polar DHETs (retention times of 12 to 15 min). This result confirms the initial observation obtained using liver microsomes (Fig. 1) and illustrates the enzymatic nature of the reaction catalyzed by the partially purified cytosolic fraction. Interestingly, one fraction associated with the epoxides (retention time of 23.4 min) seemed relatively resistant to hydration by this enyzme.

The enzymatic hydrolysis of synthetic 14,15 epoxyeicosatrienoic acid. All four monoepoxides (EETs) were synthesized from radiolabeled arachidonic acid (see Materials and Methods) and tested with liver microsomes and cytosol to determine the extent of hydrolysis to vic-dihydroxy compounds (DHETs). An example of these studies is shown in Fig. 3 (Curves A and B) where the 14,15-EET was used. Incubation of synthetic 14,15-epoxyeicosatrienoic acid (50 μM final concentration) with liver microsomes for 10 min (Fig. 3, Curve A) resulted in only a small (but detectable) increase in the formation of a radioactive metabolite with an HPLC retention time identical to authentic 14,15-DHET. In contrast, incubation of this synthetic epoxide with liver cytosol (Fig. 3, Curve B) showed the formation of a many-fold larger amount of this same metabolite.

Comparable studies using only the partially purified epoxide hydrolase in buffer with the synthetic epoxide were not successful since a high background rate of "spontaneous" epoxide hydrolysis was noted. It was then determined that the presence of a small amount of lipid, either membrane bound such as that present in microsomes or the cytosolic fraction, or exogenously added as dilauroylglycero-3-phosphocholine to the buffer system, stabilized the synthetic epoxide.

Of interest is the observation that the synthetic epoxide was *not* hydrated to the corresponding dihydroxy acid when incubated with liver microsomes and NADPH. Indeed, only a small amount of further oxidative metabolism of the epoxide was obtained during 10 min of incubation. It was noted, however, that the dihydroxy derivative formed following hydration by the

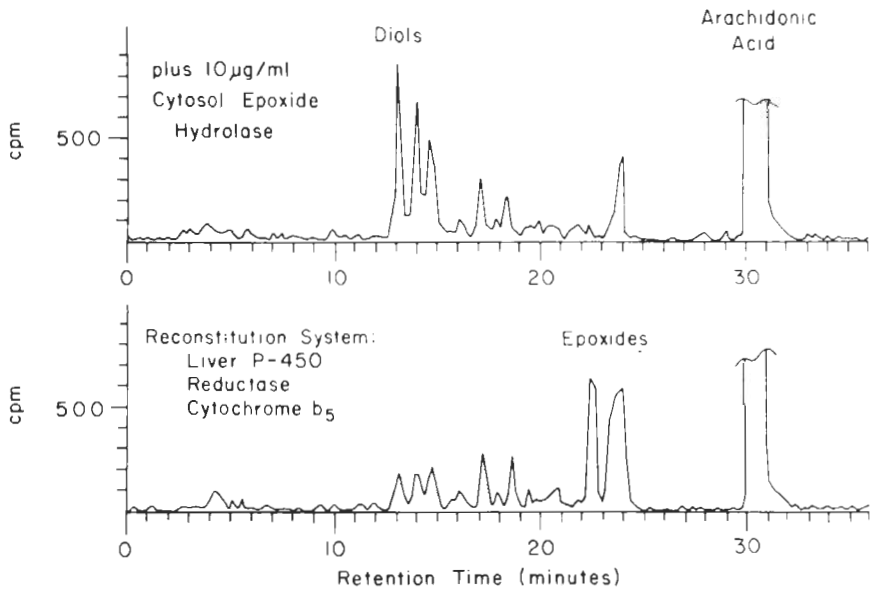

FIG. 2. The effect of partially purified cytosolic epoxide hydrolase on the balance of metabolites formed using a reconstituted cytochrome *P*-450-containing electron transport system. An aliquot containing 2 nmol of cytochrome *P*-450, was mixed with 2 nmol of purified NADPH-cytochrome *P*-450 reductase, and 2 nmol of purified rat liver cytochrome b_5 and 200 μg of dilauroylglycero-3-phosphocholine. After 5 min incubation at room temperature the samples were diluted to 2 ml with a buffer mixture containing an NADPH-generating system. [1-^{14}C]Arachidonic acid was added to give a final concentration of 100 μM. The reaction was initiated by the addition of 0.3 mM (final concentration) of NADPH. After 5 min incubation at 25°C the reaction was stopped by addition of ethyl acetate containing acetic acid as described under Materials and Methods. In the upper tracing the reaction mixture was supplemented by the addition of 10 μg/ml of purified cytosolic epoxide hydrolase.

epoxide hydrolase was further oxidatively metabolized (unpublished results).

The enzymatic hydrolysis of synthetic epoxides with cytosolic epoxide hydrolase. After establishing the conditions required to stabilize the synthetic epoxides, by utilizing sonicated dispersions of dilauroylglycero-3-phosphocholine, a series of experiments were carried out to measure the rate of catalysis and the enzyme affinity for the synthetic 14,15-EET. As shown in Fig. 4 (Curve A), the synthetic 14,15-EET is rapidly hydrated to the corresponding vic-diol in an enzyme concentration-dependent manner. When a series of comparable experiments were carried out using a sample of purified liver microsomal epoxide hydrolase (kindly provided by Professor Franz Oesch of Mainz, Germany), essentially *no* conversion of the 14,15-EET to a vic-diol was observed.

The epoxide hydrolase activity of the partially purified cytosolic fraction demonstrates a relatively low apparent K_m (8 μM) for the 14,15-EET and a maximum velocity of about 1400 nmol/min/mg protein. These data indicate that the hydrolase activity isolated from the cytosol shows specificity for these arachidonic acid epoxides over all other substrates so far examined. This apparent specificity suggests that such epoxides may be one type of endogenous substrate for this enzyme.

Studies with synthetic 5,6-, 8,9-, and 11,12-epoxyeicosatrienoic acids. Satisfied that the 14,15-EET was a good substrate for the partially purified cytosolic epoxide hydrolase, the metabolism of the other three epoxides formed during the NADPH-dependent oxidation of arachidonic acid by liver microsomal cytochrome *P*-450 was studied. In particular, attention was directed

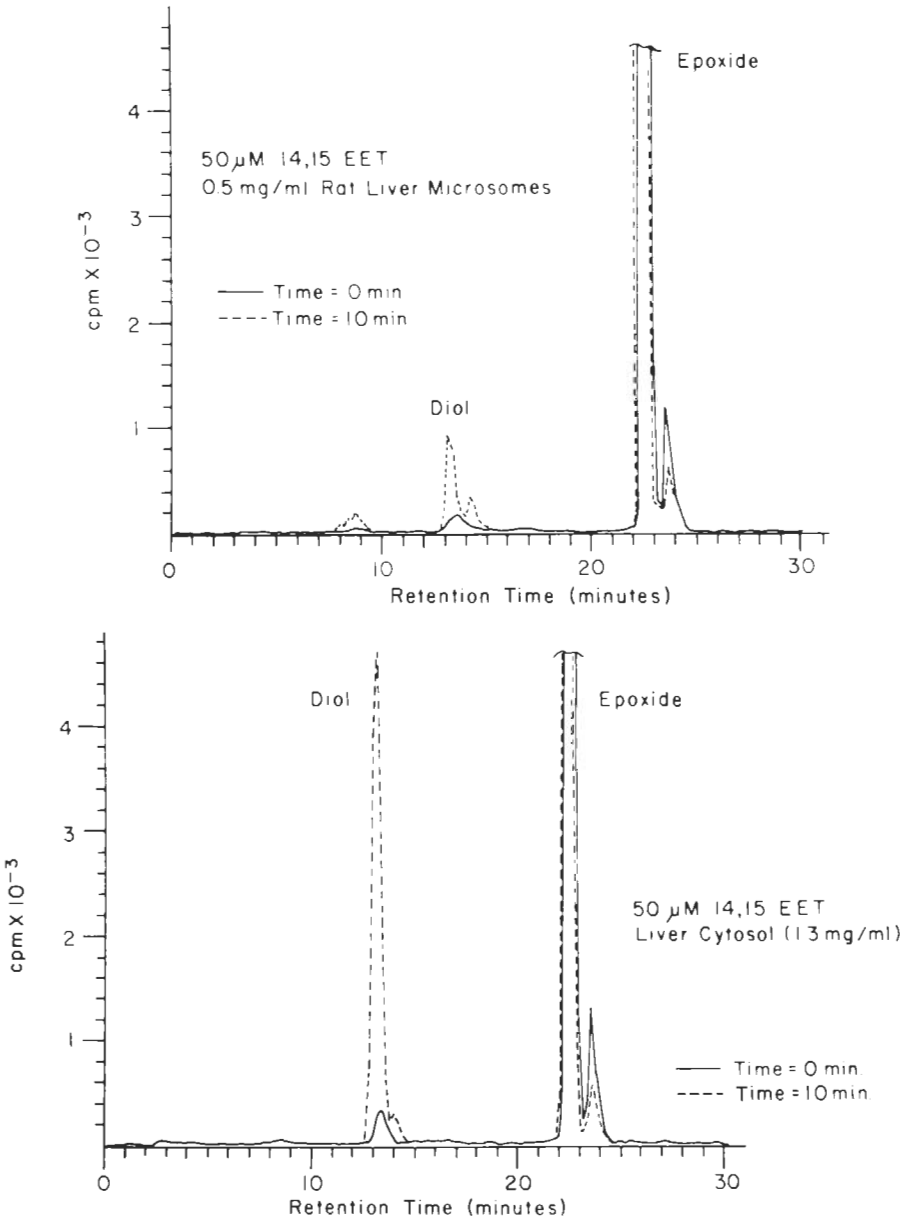

FIG. 3. The hydrolysis of synthetic 14,15-epoxyeicosatrienoic acid by rat liver cytosol. An aliquot of synthetic [1-^{14}C]-14,15-EET was added to give a final concentration of 50 μM to 2 ml of a suspension of rat liver microsomes (final concentration 0.5 mg/ml) (Upper Curve) or rat liver cytosol (final concentration 1.3 mg/ml) (Lower Curve). The samples were extracted with acidified ethyl acetate at time = 0 (solid line curves) or after 10 min incubation (dashed line curves). The samples were analyzed by HPLC as described under Materials and Methods. The small amount of radioactivity appearing at a retention time of 24 min represents an unknown contaminant in the synthesized 14,15-EET.

toward understanding the nature of the metabolite which appeared to be only slowly reactive with the partially purified cytosolic epoxide hydrolase in the studies using the reconstituted cytochrome *P*-450 containing reaction system (see Fig. 2,

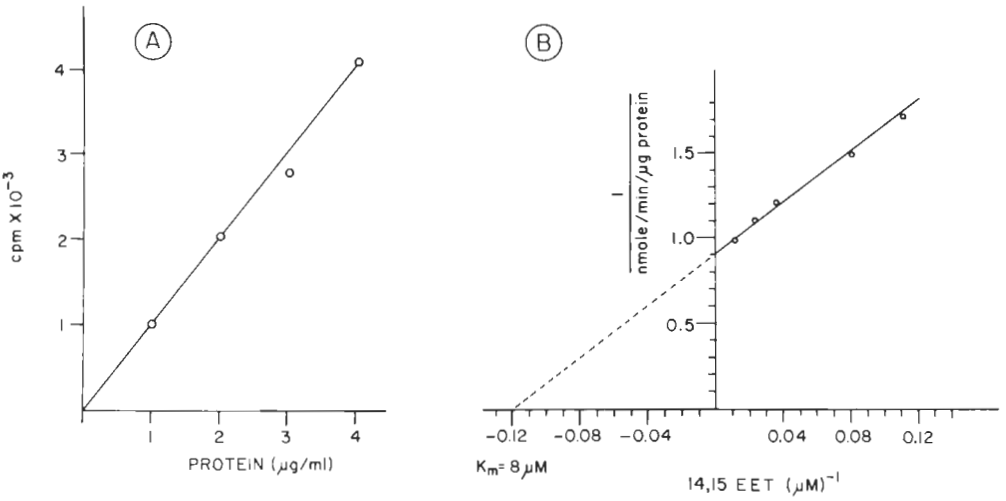

FIG. 4. Kinetic characteristics of the partially purified cytosolic epoxide hydrolase for the hydration of synthetic 14,15-EET. A sonicated suspension of dilauroylglycero-3-phosphocholine was diluted in 50 mM Tris-Cl (pH 7.5), 150 mM KCl, and 10 mM $MgCl_2$ buffer to a concentration of 100 μg/ml. The designated concentration of [1-^{14}C]-14,15-EET was added and the sample allowed to incubate at 25°C for 1 min (B). Varying concentrations of mouse liver cytosolic epoxide hydrolase were added (A) and the reaction terminated after 2 min by the addition of acidified ethyl acetate. The sample was analyzed by HPLC as described under Materials and Methods and the amount of product was determined.

fraction with a retention time of 23.9 min). As shown in Table IV, both the 8,9-and 11,12-EETs were suitable substrates for conversion to their respective vic-diols when incubated with the partially purified mouse liver cytosolic epoxide hydrolase, using experimental conditions comparable to those described above for the studies carried out with the 14,15-EET. It should be noted that the hydration rates of these epoxy acid derivatives are significantly greater than those reported by Oliw *et al.* (4) in their studies with the purified *microsomal* epoxide hydrolase. No efforts were made in the present study to optimize the conditions of the reaction, i.e., only a 50 μM substrate concentration was tested for the experiments summarized in Table IV. Of interest is the very low activity noted when the 5,6-epoxyeicosatrienoic acid was used as substrate. The low reactivity of the synthetic 5,6-EET is of possible significance because of its demonstrated biological activity affecting the *in vitro* release of several peptide hormones (9, 10).

DISCUSSION

The importance of epoxide hydrolase in the metabolism of many xenobiotic metabolites (oxiranes), formed during the oxidative reaction catalyzed by cytochrome *P*-450, is well established (5). Considerable effort has been invested in the isolation, purification, and characterization of the enzyme predominantly associated with the membrane structure of liver microsomes (6, 7). A second epoxide hydrolase, a higher molecular weight, soluble form present primarily in the cytosol, has recently been described (21). Gill *et al.* (22, 23) first identified the role of a cytosolic epoxide hydrolase from mouse liver and kidney in the metabolism of juvenile hormones and other terpenoid epoxides (8). This enzyme possesses significantly different properties and substrate specificities when compared with the membrane-bound enzyme isolated from liver microsomes (8, 24). In particular, the soluble cytosolic enzyme was effective in the hydration of mono-substituted oxi-

TABLE IV

COMPARISON OF THE RATES OF HYDRATION OF THE EPOXIDES OF ARACHIDONIC ACID BY THE PARTIALLY PURIFIED CYTOSOLIC EPOXIDE HYDROLASE OF MOUSE LIVER

EET	Rate (μmol/min/mg protein)
5,6	0.069[a]
8,9	0.37
11,12	0.64
14,15	1.26

Note. [1-^{14}C]-EETs, at an initial concentration of 50 μM, were incubated with 0.1 mg/ml of dilauroylglycero-3-phosphocholine for 5 min at 25°C. The reaction was initiated by the addition of 10 μg of partially purified mouse liver cytosolic epoxide hydrolase and stopped by addition of ethyl acetate. Products were analyzed as described under Materials and Methods. Samples were taken at 1, 2, and 5 min to insure the linearity of the reaction (except for the 5,6-EET where a sample at 10 min was also taken).

[a] This rate represents the sum of both the DHET formed as well as the appearance of the δ-lactone of 5,6-DHET.

ranes as well as some *cis*- and *trans*-, di-, tri-, and tetrasubstituted aliphatic epoxides, e.g., the conversion of *cis*- and *trans*-epoxystearates or their methyl esters to the corresponding *vic*-diol products. Of interest is the potential role of this cytosolic epoxide hydrolase in attenuating a positive response when the S-9 fraction of liver is used in the Ames test to evaluate the mutagenicity of chemicals in the Salmonella assay (25).

Recently our laboratory has focused attention on the role of liver microsomal cytochrome *P*-450 as an epoxygenase for the oxidative metabolism of polyunsaturated fatty acids (arachidonic acid) to a variety of products (1). Studies with either the microsomal-bound cytochrome *P*-450 or with a reconstituted system containing purified components of the microsomal electron transport chain have shown that the predominant metabolities formed are novel epoxides of arachidonic acid (2). These epoxy acids are relatively stable in the presence of lipid and accumulate during the aerobic steady state of the reaction. Comparable studies on the cytochrome *P*-450-directed metabolism of arachidonic acid by Oliw *et al.* (3, 4), using either kidney or liver microsomes isolated from rabbits, reported the formation of vic-dihydroxyeicosatrienoic acids unless the epoxide hydrolase inhibitor, 1,2-epoxy-3,3,3-trichloropropane, was added to the incubation mixture. Only in the presence of this inhibitor, clear evidence for the formation of epoxy acid derivatives was obtained. These results differ from those reported earlier by us (2) and may be explained by the presence of significant amounts of a contaminating cytosolic epoxide hydrolase in the preparation of microsomes used by Oliw *et al.* (3) or the result of significantly different experimental conditions of analysis, i.e., incubation of the reaction system for 15 min at 37°C and at a different pH, i.e., pH 8.7. Such differences may also explain their observation that incubation of synthetic epoxides with either liver or kidney microsomes (as well as a cytosolic fraction from these tissues) results in hydration of these epoxides. Previous studies have indicated the presence of a tightly bound microsomal epoxide hydrolase which has many of the properties of the cytosolic enzyme (8, 21–23). Guenthner *et al.* (26) recently provided data supporting the concept that there is a minor but potentially important microsomal form of the cytosolic epoxide hydrolase. Possibly this activity of the microsomal form led Oliw *et al.* (3) to overestimate its total contribution to the metabolism of EETs. More difficult to explain is the report (4) that incubation of synthetic arachidonic acid epoxides with a purified rat liver microsomal epoxide hydrolase results in the hydration of these compounds. Comparison of their kinetic data with results described in this paper shows that the partially purified *cytosolic* epoxide hydrolase is far more active than the purified *microsomal* epoxide hydrolase.

Of special interest is the observation of a lower reactivity of the 5,6-EET to enzymatic hydration (Table IV). Recent experiments have suggested a possible phys-

iological role for this epoxide as a potent and selective mediator for the release of somatostatin and luteinizing hormone from *in vitro* incubations of median eminence fragments and anterior pituitary cells, respectively (9, 10).

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