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Regio- and Enantiofacial Selectivity of Epoxyeicosatrienoic Acid Hydration by Cytosolic Epoxide Hydrolase*

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The hydration of *cis*-epoxyeicosatrienoic acids to the corresponding *vic*-dihydroxyeicosatrienoic acids by cytosolic epoxide hydrolase demonstrates moderate regioselectivity with rates of hydration highest for the 14,15-epoxide and lower for the 11,12- and 8,9-epoxide (4.5, 1.6, and 1.5 μmol of product/mg of protein/min, respectively). Incubations of the 8,9- and 14,15-epoxides with cytosolic epoxide hydrolase show stereoselective formation of diols (7:3 and 4:1 ratio of antipodes, respectively) and concomitant chiral enrichment of the remaining unmetabolized substrate. In contrast, hydration of the 11,12-epoxide is nonenantioselective. The K_m value of the enzyme for the 14(*R*),15(*S*)-epoxide is 3 μM. Incubations of the enantiomerically pure 8,9- and 14,15-epoxides with lung or liver cytosol, followed by chiral analysis of the resulting diols demonstrate selective cleavage of the oxirane ring at C_9 and C_{15}, respectively. On the other hand, cleavage of the 11,12- oxirane ring was less selective. The stereochemical preference of the cytosolic epoxide hydrolase, together with the known chiral composition of the endogenous arachidonate epoxide pools, suggests a functional role for this enzyme in the metabolism of these important compounds.

The role of cytochrome P-450 in the NADPH-dependent oxygenation of arachidonic acid is well established (1–3). Oxidative metabolism by the hemeprotein leads to the formation of several tissue-specific products, including 5,6-, 8,9-, 11,12-, and 14,15-EET[1] (4). Chiral analysis of rat liver microsomal arachidonic acid epoxygenase metabolites reveals their enantioselective formation (5) and that this enantioselectivity is under cytochrome P-450 isoform control (5). Additionally, the asymmetric nature of the endogenous EETs in liver (6, 10), lung (7), kidney (8), and plasma (9) confirms the biosynthetic origin of 14(*R*),15(*S*)-, 11(*S*),12(*R*), and 8(*S*),9(*R*)-EET (10). Recent data show that the EETs are found esterified to the *sn-2* position of glycerophospholipids and that acylation is stereoselective with preference for these same endogenous EET enantiomers (11). The EETs have been shown to exhibit potent biologic activities, some of which can be stereospecific (12–15).

The EETs are enzymatically hydrated to the corresponding DHETs by epoxide hydrolases (16–18). A functional role for the cEH in the catalysis of EET hydration was proposed based on its relatively low apparent K_m for the EETs and its high maximal velocity of hydration (16). Moreover, activity comparisons demonstrated that the cytosolic enzyme was more active than the microsomal-bound one for these substrates (16). The DHETs are also produced *in vivo* (19) and have been shown to alter the hydroosmotic responses of the toad bladder and the rabbit cortical collecting tubule to vasopressin (20, 21). Significantly, the urinary excretion of the DHETs is increased during pregnancy-induced hypertension (19).

Despite extensive study (22), the functional significance of the cEH is largely unknown. A number of investigators have proposed a role for the cEH in the hydration and detoxification of potentially harmful xenobiotics (23–25). Although several endogenously occurring epoxides can serve as substrates for the cEH *in vitro* (16, 17, 26), the role of this enzyme in the *in vivo* metabolism of endogenous epoxides is unknown. Utilizing established methodology for the chiral analysis of EETs (27) and DHETs (28), we report the enantioselective hydration of EETs by rabbit liver and lung cytosol. Additionally, we utilize a purified cEH to confirm its role in the catalysis of EET hydration by cytosol. Based on this analysis and on the known chiral composition of endogenous EET pools, we suggest a role for this enzyme in the *in vivo* metabolism of EETs to DHETs and in the control of the steady state concentration of these important compounds.

MATERIALS AND METHODS

Isolation of Cytosolic and Microsomal Fractions—Male New Zealand White rabbits (3 kg) were maintained at 22 °C, with alternating cycles of light and darkness, and fed *ad libitum* Purina Rabbit Chow and water. Rabbits were sacrificed by lethal intravenous injection of pentobarbital (400–500 mg), and their livers and lungs were perfused *in situ* with ice-cold 0.15 M KCl containing 1 mM EDTA. The organs were removed, weighed, minced, washed twice with the perfusing solution, and immediately homogenized in 0.01 M Tris-Cl buffer (pH 7.5) containing 0.25 M sucrose (20 g of wet tissue/100 ml). Cytosolic and microsomal fractions were obtained by differential centrifugation at 4 °C as described (29). The 105,000 × *g* supernatant was used as the cytosolic fraction. Microsomal fractions were resuspended in 0.15 M KCl, centrifuged at 105,000 × *g*, and resuspended in 0.01 M Tris-Cl buffer (pH 7.5) containing 0.25 M sucrose (4–5 mg of protein/ml).

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[1] The abbreviations used are: EET, *cis*-epoxyeicosatrienoic acid; DHET, *vic*-dihydroxyeicosatrienoic acid; Me, methyl; HPLC, high pressure liquid chromatography; cEH, cytosolic epoxide hydrolase.

Microsomal fractions were maintained at 4 °C and used within 48 h of preparation. Cytosolic fractions were maintained at −80 °C for up to 6 months. Protein determination was performed using the Bio-Rad protein assay.

Synthetic Procedures—Racemic EETs were prepared as previously described (30, 31). The enantiomers of 14,15- and 8,9-EET were prepared by total asymmetric synthesis according to published procedures (32, 33). DHETs and [1-^{14}C]DHETs (50–55 μCi/μmol) were prepared by chemical hydration of individual EETs as follows. Samples (10–100 μg) were dissolved in 25–50 μl of CH_3CN and combined with 1 ml of CH_3CO_2H/H_2O (1:1). After 12 h at 45 °C under an argon blanket and with constant mixing, 1 ml of 1 M Tris-Cl buffer (pH 7.5) was added, and the resulting DHETs were extracted into ethyl ether. All synthetic EETs and DHETs were purified by reverse-phase HPLC on a μBondapak C_{18} column (4.6 × 300 mm, Waters Associates, Milford, MA) using a linear solvent gradient from $CH_3CO_2H/H_2O/CH_3CN$ (0.1:49.95:49.95) to CH_3CO_2H/CH_3CN (0.1:99.9) over 40 min at 1 ml/min. Samples were collected based on their absorbance at 210 nm, dried under a nitrogen stream, and stored at −80 °C under an argon blanket. [1-^{14}C]EET and [1-^{14}C]EET-Me standards were synthesized from either [1-^{14}C]arachidonic acid or [1-^{14}C]methyl arachidonate (52–55 μCi/μmol) by nonselective epoxidation (34). Enantiomerically pure [1-^{14}C]EETs were obtained by chiral-phase HPLC separation of racemic [1-^{14}C]EETs (27). Methylations were performed using an ethereal solution of diazomethane, as previously described (35). Pentafluorobenzyl esters were prepared as previously described (10). Trimethylsilyl ethers were prepared using 25% (v/v) bis(trimethylsilyl)trifluoroacetamide in anhydrous pyridine (36). Saponifications were performed as described in (34). All derivatized compounds were purified by reverse-phase HPLC prior to use.

Chiral-phase HPLC Analysis—The optical isomers of 14,15-EET-Me and of 8,9- and 11,12-EET pentafluorobenzyl ester were resolved by chiral-phase HPLC on either a Chiralcel OB or OD column (4.6 × 250 mm, J. T. Baker Chemical Co.) as previously described (27). Fractions were collected based on their absorbance at 210 nm or by utilizing a fraction collector and liquid scintillation. Absolute configurations were assigned as described (27). The optical isomers of 8,9-, 11,12-, and 14,15-DHET-Me were also resolved by chiral-phase HPLC on either a Chiralcel OC or OD column (4.6 × 250 mm, J. T. Baker Chemical Co.) as described (28). Absolute configurations were assigned as described in Ref. 28. Optimal resolution required extensive column equilibration with the mobile phase for both EETs and DHETs.

Cytosolic and Microsomal Incubations—Lung or liver cytosol (1.0 and 0.2 mg of protein/ml, respectively) in 150 mM KCl, 10 mM $MgCl_2$, and 50 mM Tris-Cl buffer (pH 7.5) was equilibrated at 30 °C with constant mixing. Reactions were initiated by the addition of [1-^{14}C] EET (0.8–1.2 μCi/μmol, 10 mM in ethanol) to a final concentration of 100 μM. Aliquots were removed at designated points in time, and the reaction products were extracted into diethyl ether, dried under a nitrogen stream, and separated by reverse-phase HPLC on a 5-μm Dynamax Microsorb C_{18} column (4.6 × 250 mm, Rainin Instruments Co., Woburn, MA) using the solvent program described above. The reaction products were quantified by on-line liquid scintillation using a Radiomatic Flo-One β-Detector (Radiomatic Instruments, Tampa, FL). For chiral analysis, fractions were collected every 30 s, and the radioactivity of 5% aliquots from each fraction were assessed by liquid scintillation. Fractions with retention times corresponding to DHETs (15–19 min) and EETs (25–29 min) were pooled and dried under a nitrogen stream. For chiral analyses, reactions were terminated after approximately 25% metabolism. Initial velocities are expressed in nanomoles of product formed/min/mg of protein. Proteins were denatured at 100 °C for 10 min.

Preparation of Purified Cytosolic Epoxide Hydrolase—Purified cEH was prepared from Swiss mouse liver cytosol as described (37, 38). The enzyme was judged homogeneous based on its electrophoretic properties (37, 38). The purified enzyme was stored in 0.1 M KH_2PO_4 buffer (pH 7.4) with 0.05% (w/v) bovine serum albumin (fraction V, Sigma) at −20 °C and was stable for approximately 3 months. A decrease in specific activity was noted with repeated freezing and thawing.

Incubations Utilizing Purified cEH—[1-^{14}C]EETs (0.8–1.2 μCi/μmol, 10 mM in ethanol) were added to a final concentration of 100 μM and preincubated with a sonicated dispersion of dilauroylglycero-3-phosphocholine (0.1 mg/ml) in 150 mM KCl, 10 mM $MgCl_2$, and 50 mM Tris-Cl buffer (pH 7.5) for 5 min at 30 °C. The reaction was initiated by the addition of purified cEH (final concentration, 0.001 mg of protein/ml), and the reaction products were analyzed as described earlier. For K_m determinations, the concentration of EET was varied between 0.5 and 100 μM.

RESULTS AND DISCUSSION

Incubations of [1-^{14}C]14,15-EET with rabbit liver and lung cytosol or liver and lung microsomes revealed a 10–14-fold higher rate of DHET production in the cytosolic fractions relative to the microsomal fractions (Table I). Under our experimental conditions and at difference with allylic epoxides (39), the EETs do not show detectable nonenzymatic hydration. Thus, in incubations using heat-denatured liver or lung cytosol, we were unable to measure significant DHET formation. These results suggested that, as shown for rat liver (16), enzymatic EET hydration occurred predominantly in the cytosolic fraction. Furthermore, on a per milligram protein basis, liver cytosol was approximately 10-fold more active than lung cytosol.

Regio- and Enantioselectivity of Epoxide Hydration by Cytosol—EET hydration by liver and lung cytosol was moderately regioselective, with 14,15-EET exhibiting the highest rates (Table II). Compared with 14,15-EET, the hydration of 8,9-EET was slightly slower, whereas that of 11,12-EET was markedly slower. Chacos *et al.* (16) similarly observed that 14,15-EET was rapidly hydrated by rat liver cytosol. The enzymatic hydration of 5,6-EET was difficult to evaluate due to its lability. On the other hand, derivatization to the corresponding methyl ester prevented intramolecular protonation and stabilized the 5,6-oxirane ring. Incubations of 5,6-EET-

TABLE I

Hydration of 14,15-EET by rabbit liver and lung cytosol or microsomes

Rabbit liver cytosol (0.2 mg of protein/ml), liver microsomes (1.0 mg of protein/ml), lung cytosol (1.0 mg of protein/ml), or lung microsomes (2.0 mg of protein/ml) were incubated with [1-^{14}C]14,15-EET (100 μM) at 30 °C. Hydration rates were determined as described under "Materials and Methods." Values given are averages from at least four different experiments with S.E. <5% of the mean.

Organ	Subcellular fraction	Hydration rate
		nmol DHET/mg protein/min
Liver	Cytosol	22.6
	Microsomes	1.6
Lung	Cytosol	1.9
	Microsomes	0.2

TABLE II

Regio- and enantioselectivity of EET hydration by rabbit liver and lung cytosol

Rabbit liver cytosol (0.2 mg of protein/ml) or lung cytosol (1.0 mg/ml) were incubated with each [1-^{14}C]EET regioisomer (100 μM) at 30 °C. Hydration rates were determined as described under "Materials and Methods" and are averages of at least four different experiments with S.E. <10% of the mean. For product stereoselectivity determinations, DHETs were derivatized to the corresponding methyl esters and purified by reverse-phase HPLC prior to chiral-phase HPLC analysis.

Organ	Regioisomer	Hydration rate	DHET enantiomer produced	
			R,R	*S,S*
		nmol DHET/mg protein/min	%	
Liver	14,15-EET	22.6	79	21
	11,12-EET	6.8	55	45
	8,9-EET	16.8	30	70
Lung	14,15-EET	1.9	80	20
	11,12-EET	0.5	57	43
	8,9-EET	1.3	33	67

Me with liver or lung cytosol and analysis by reverse-phase HPLC resulted in the appearance of several fractions, labeled *A-D* (Fig. 1). Based on (i) comparisons of their retention times with those of synthetic standards, (ii) susceptibility to saponification, and (iii) gas chromatography/mass spectrometry evidence (36), it was demonstrated that fraction A contained the δ-lactone of 5,6-DHET, fraction B contained 5,6-EET, and fraction C contained 5,6-EET-Me. We concluded, based on the relative proportions of A, B, and C (A:B:C = 1:3:5) and on the enzymatic origin of fraction B, that (i) cytosol contained potent 5,6-EET-Me esterase activity, (ii) the 5,6-EET product (Fig. 1*B*) underwent primarily chemical hydration to the corresponding DHET, (iii) the DHET rearranged rapidly to form the δ-lactone of 5,6-DHET (Fig. 1*A*), and (iv) enzymatic hydration of either 5,6-EET or 5,6-EET-Me was negligible. Attempts to inhibit the esterase with bis(4-nitrophenyl) phosphate were unsuccessful. The structure of the radioactive material in fraction D remains undetermined.

The enantioselective behavior of the microsomal epoxide hydrolase toward a number of xenobiotic substrates has been studied in detail (40–42). Considerably less is known about the stereoselectivity of the cytosolic enzyme. Haeggstrom and coworkers (39) reported the enantioselective hydration of (5*S*)-*trans*-5,6-oxido-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid to (5*S*,6*R*)-dihydroxy-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid by human liver cEH. More recently, Miki *et al.* (43) reported that (11*S*)-*trans*-11,12-oxido-5,14-*cis*-7,9-*trans*-eicosatetraenoic acid was stereoselectively hydrated to (11*R*,12*S*)-dihydroxy-5,14-*cis*-7,9-*trans*-eicosatetraenoic acid by guinea pig liver cEH. Incubations of 8,9- and 14,15-EET with rabbit liver or lung cytosol demonstrated stereoselective formation of 8,9- and 14,15-DHET in a 7:3 and a 4:1 ratio of antipodes, respectively (Table II). Enantioselective DHET formation was accompanied by chiral enrichment of the remaining, unmetabolized substrates with preferential hydration of 14(*R*),15(*S*)- and 8(*S*),9(*R*)-EET. In contrast, the hydration of 11,12-EET by rabbit liver or lung cytosol was nonstereoselective. Importantly, the enantioselectivity of cEH for the biologically important enantiomers (*i.e.* 14(*R*),15(S)- and 8(*S*),9(*R*)-EET) suggests a functional role for this enzyme in the *in vivo* catalysis of EET hydration.

Incubations of enantiomerically pure EETs with liver or lung cytosol demonstrated that the hydration rates for 14(*R*),15(*S*)- and 14(*S*),15(*R*)-EET were similar. In contrast, 8(*S*),9(*R*)- and 11(*S*),12(*R*)-EET were metabolized 2.4- and 6.7-fold faster than their corresponding enantiomers, respectively. These results suggested that additional factors may be important in the control of the asymmetry of diol formation

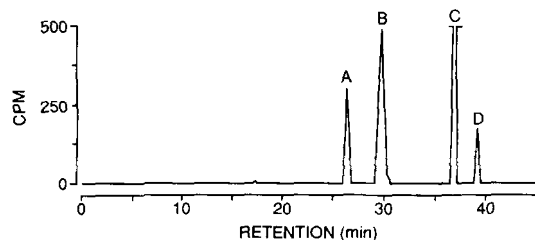


FIG. 1. **Reverse-phase HPLC chromatogram of metabolites generated by incubation of 5,6-EET-Me with rabbit lung cytosol.** Rabbit lung cytosol (1.0 mg of protein/ml) was incubated with [1-^{14}C]5,6-EET-Me (100 μM) at 30 °C for 15 min. The reaction products were extracted with diethyl ether and analyzed and quantified by reverse-phase HPLC as described under "Materials and Methods." *Ordinate*, radioactivity in counts/min; *abscissa*, retention time in minutes.

and led us to examine the regiochemistry of EET epoxide ring opening.

Regiochemistry of Oxirane Hydration—It is widely accepted that the microsomal and cytosolic epoxide hydrolases catalyze the *trans*-addition of water across the oxirane ring with inversion of configuration at the site of addition (44–46). Despite considerable investigation, the molecular topography of the cEH active site and the mechanisms responsible for oxirane ring activation are unknown (47–49). Most of the experimental evidence supports a general base-catalyzed nucleophilic addition of water to the oxirane ring by the microsomal enzyme (48, 50–52). Studies using the microsomal epoxide hydrolase have shown that water is added to the less sterically hindered oxirane carbon atom (52–54). A similar mechanism may be operative with the cytosolic enzyme (55, 56), although this enzyme has not been studied as extensively.

By chromatographic comparisons with synthetic standards, it was demonstrated that hydration of EETs proceeded exclusively by an S_N2 mechanism, affording only *threo*-diols. Given the *trans*-addition of water across the oxirane ring and retention of configuration at the unbroken carbon-oxygen bond, the relative proportion of DHET enantiomers produced during hydration of enantiomerically pure EETs will reflect the corresponding sites of epoxide ring opening and water addition (Fig. 2). For example, using 14(*S*),15(*R*)-EET, if addition occurs only at the C_{14} position (Fig. 2*A*, *path a*), then only 14(*R*),15(*R*)-DHET will be formed. Alternatively, if addition occurs only at the C_{15} position (Figure 2*A*, *path b*), then only 14(*S*),15(*S*)-DHET will be produced. If addition occurs indiscriminately, then both DHET enantiomers will be formed, and their ratio will reflect the regioselectivity of water addition. A similar analysis for 14(*R*),15(*S*)-EET is shown in Fig. 2*B*.

Incubations of [1-^{14}C]14(*R*),15(*S*)-EET with either liver or lung cytosol followed by chiral resolution of the resulting DHET enantiomers resulted in the preferential production of 14(*R*),15(*R*)-DHET (the ratio of antipodes is 6:1 and 7:1 for liver and lung cytosol, respectively), demonstrating that enzymatic oxirane ring opening was highly selective for addition at C_{15}. On the other hand, enzymatic hydration of 14(*S*),15(*R*)-EET resulted in the preferential production of 14(*S*),15(*S*)-DHET (the ratio of antipodes is 3:1 for both liver and lung cytosol), indicating a similar but less pronounced selectivity for addition at C_{15} and showing that the cEH interacts differently with these EET enantiomers. By comparison, nonenzymatic, acid-catalyzed hydration of the individual 14,15-EET enantiomers demonstrated a selectivity similar to that observed during the enzymatic hydration of 14(*S*),15(*R*)-EET (the ratio of antipodes is 3:1). Dietze *et al.* (57) recently studied the incorporation of ^{18}O-labeled water during cEH catalyzed hydrolysis of the 2,3-epoxy-3-(4-nitrophenyl) glycidol enantiomers. They found that with both enantiomers, although the incorporation of ^{18}O occurred in either carbon atom of the epoxide ring, only the (2*S*,3*S*)-epoxide demonstrated a preferred binding orientation. Their data suggest, as does ours, that the stereochemistry of the substrate influences the regiochemistry of water addition by the cEH.

Incubations of 8(*R*),9(*S*)-EET with either liver or lung cytosol resulted in the preferential production of 8(*R*),9(*R*)-DHET (the ratio of antipodes is 3:1 for both liver and lung cytosol), showing enzyme selectivity for hydration at C_9 (Fig. 3). Enzymatic hydration of 8(*S*),9(*R*)-EET resulted in the preferential production of 8(*S*),9(*S*)-DHET (the ratio of antipodes is 4:1 for both liver and lung cytosol) indicating a similar selectivity for hydration at C_9 (Fig. 3). In contrast, the

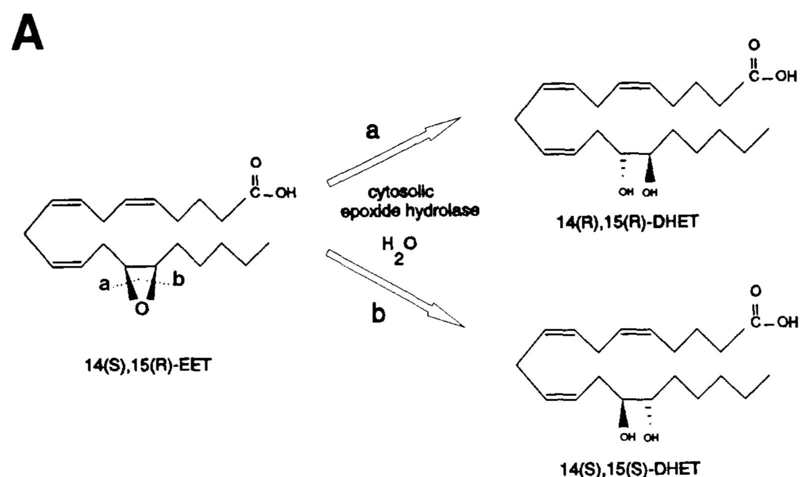
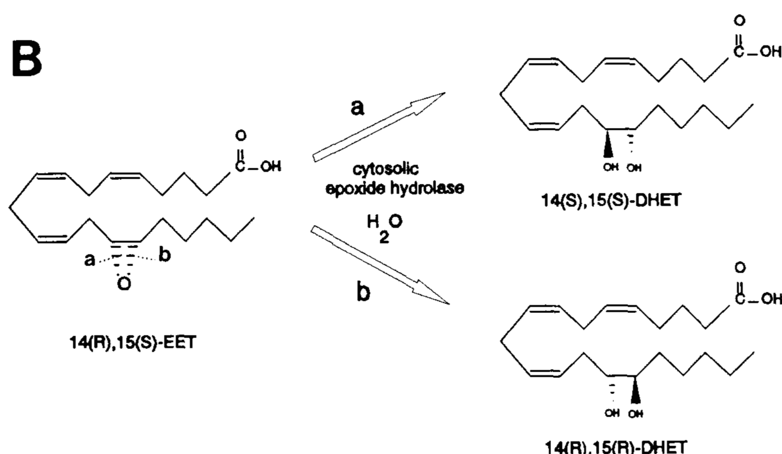


FIG. 2. **Regiochemistry of oxirane ring opening.**



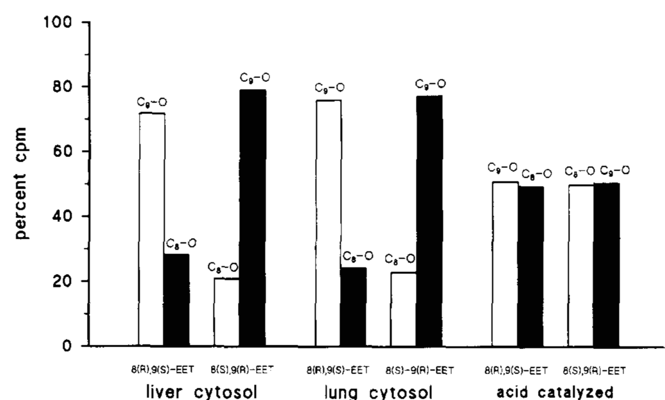


FIG. 3. **Regiochemistry of 8,9-EET ring opening.** Rabbit liver cytosol (0.2 mg of protein/ml) or lung cytosol (1.0 mg of protein/ml) was incubated with enantiomerically pure [1-^{14}C]8(R),9(S)- or 8(S),9(R)-EET (100 μM) at 30 °C. The reactions were terminated by the addition of diethyl ether, and the organic soluble metabolites were analyzed as described under "Materials and Methods." Values shown are the averages of at least two different experiments and differed from each other by less than 5%. *Open bars*, 8(R),9(R)-DHET enantiomer; *solid bars*, 8(S),9(S)-DHET enantiomer.

nonenzymatic, acid-catalyzed hydration of the 8,9-EET enantiomers yielded racemic DHETs (Fig. 3). The high enantioselectivity of epoxide hydration in conjunction with the increased enzymatic selectivity for addition at C_{15} (in the case of 14(R),15(S)-EET) and the marked differences in the regiochemistry of epoxide ring opening between the enzymatic and the chemical hydration (in the case of the 8,9-EET enantiomers) show that the cEH binds these substrates in a relatively rigid conformation, allowing regioselective destabilization of the oxirane ring and favoring the regioselective and asymmetric nucleophilic addition of water.

Incubation of 11(R),12(S)-EET with liver cytosol resulted in the formation of 11(R),12(R)- and 11(S),12(S)-DHET in a 2:1 ratio of antipodes. On the other hand, enzymatic hydration of 11(S),12(R)-EET yielded racemic products. In a similar fashion, the chemical hydration of homochiral 11,12-EET samples generated achiral DHETs. As mentioned, the enzymatic hydration of 11,12-EET yielded racemic DHETs (Table II) in spite of the fact that the individual enantiomers were metabolized at significantly different rates. Subsequent analysis of the chirality of the unmetabolized substrate demonstrated that, under our experimental conditions, both EET enantiomers were metabolized with equal efficiency (*i.e.* the remaining 11,12-EET was racemic). We conclude that for 11,12-EET, the lack of enantioselectivity is probably due to poor regioselectivity of water addition and the comparable efficiency of the enzyme for both EET enantiomers.

Studies with a Purified cEH—Crude enzyme preparations are an important tool for the initial characterization of an

enzymatic pathway. However, due to the presence of multiple enzymes and/or binding proteins in these crude preparations and the potential influence of these factors on the observed metabolism, it is both necessary and important to validate such results using a purified enzyme preparation. In order to confirm the role of the cEH in the catalysis of EET hydration by the cytosolic fractions, we utilized an electrophoretically homogeneous preparation of cEH from mouse liver to study the hydration of racemic and enantiomerically pure EETs.

Incubations of each of the EET regioisomers with the pure enzyme demonstrated that epoxide hydration was rapid and regioselective, with 14,15-EET exhibiting the higher rate of hydration (Table III). The hydration of 8,9- and 11,12-EET proceeded at significantly lower rates. As mentioned, the reactivity of rabbit liver and lung cytosol toward 5,6-EET-Me was difficult to evaluate due to the presence of an active cytosolic esterase. The 5,6-EET has been reported to be active in a variety of biologic systems (13). Of interest, Chacos *et al.* (16) reported the low reactivity of cEH toward 5,6-EET. Since recent work has demonstrated the presence of 5,6-DHET in rat urine (58) and since hydration may play an important role in controlling its tissue concentration, we compared the hydration of 5,6- and 14,15-EET-Me by the pure enzyme. The metabolism of 5,6- and 14,15-EET-Me by purified cEH proceeded at comparable rates (1,198 ± 184 and 3,304 ± 419 nmol/mg of protein/min, respectively).

As with liver and lung cytosol, incubations of 8,9- and 14,15-EET with the purified cEH resulted in the stereoselective formation of DHETs (7:3 and 4:1 ratios of antipodes, respectively) (Fig. 4). The hydration of 11,12-EET by the purified enzyme yielded racemic products (Fig. 4). The stereoselectivity of 8,9- and 14,15-EET hydration was supported by demonstrating chiral enrichment of the remaining substrate with preferential hydration of 14(*R*),15(*S*)- and 8(*S*),9(*R*)-EET. Incubations of enantiomerically pure EETs with pure cEH demonstrated that the endogenous enantiomers (namely 14(*R*),15(*S*)-, 11(*S*),12(*R*)-, and 8(*S*),9(*R*)-EET) were metabolized significantly faster than their antipodes (Table III). Furthermore, a more detailed kinetic analysis of 14(*R*),15(*S*)-EET hydration revealed that the K_m of the pure enzyme for this substrate was 3 μM. Based on these data, we concluded (i) that the catalysis of EET hydration by lung and liver cytosol was mediated by the cytosolic epoxide hydrolase, (ii) that cEH rapidly and stereoselectively hydrated EETs, and (iii) that the cEH had a remarkably high affinity for 14(*R*),15(*S*)-EET.

Cytochrome P-450 catalyzes the regio- and stereospecific

TABLE III

Hydration of racemic and enantiomerically pure EETs by purified cEH

Purified cEH (1.0 μg/ml) was incubated with [1-^{14}C]EET regio- and stereoisomers (100 μM) in the presence of dilauroylglycero-3-phosphocholine (0.1 mg/ml) at 30 °C. Hydration rates were determined as described under "Materials and Methods." Values given for reaction rates are averages of at least three different expriments ± S.E.

Substrate	Hydration rate
	nmol/mg protein/min
14,15-EET	4528 ± 434
14(*R*),15(*S*)-EET	7994 ± 938
14(*S*),15(*R*)-EET	1992 ± 190
11,12-EET	1651 ± 145
11(*R*),12(*S*)-EET	401 ± 19
11(*S*),12(*R*)-EET	1356 ± 67
8,9-EET	1448 ± 178
8(*R*),9(*S*)-EET	150 ± 21
8(*S*),9(*R*)-EET	1704 ± 254

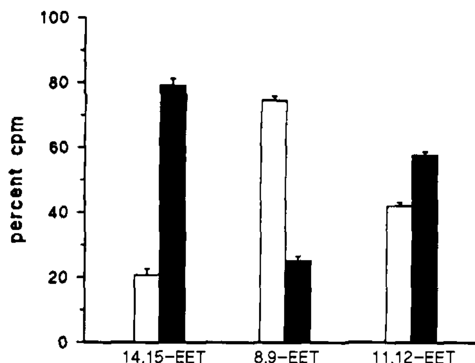


FIG. 4. **Enantioselectivity of EET hydration by purified cEH.** Purified cEH (1.0 μg/ml) was incubated with [1-^{14}C]EET regioisomers in the presence of dilauroylglycero-3-phosphocholine (0.1 mg/ml) at 30 °C. Reactions were terminated by the addition of diethyl ether, and the organic soluble metabolites were extracted. Products were separated by reverse-phase HPLC as described under "Materials and Methods." The DHETs were derivatized to the corresponding methyl esters and purified by reverse-phase HPLC prior to chiral phase HPLC analysis. Values shown are the averages of at least three different experiments ± S.E. *Open bars*, *S,S*-enantiomer; *solid bars*, *R,R*-enantiomer.

oxidation of arachidonic acid to yield a family of biologically active molecules, including hydroxyeicosatetraenoic acids, ω/ω-1-alcohols, and EETs. The findings that EETs were endogenous constituents of several tissues (6–9) and that their formation was enantioselective (5) established the arachidonate epoxygenase as a member of the arachidonic acid metabolic cascade. The asymmetric nature of the endogenous EETs (10), as well as the potent and sometimes stereospecific biological activities of these compounds (14, 15) suggested that those enzymatic processes that control the tissue concentrations of individual EET enantiomers might be important in the regulation of their functional effects. The recent description of EET-phospholipid pools and the discovery that acylation of EETs to the glycerophospholipids was stereoselective (11) provided one mechanism whereby free EET enantiomer concentrations could be regulated. An important finding here is that EET hydration by cEH is also stereoselective for 14(*R*),15(*S*)-, 11(*S*),12(*R*)-, and 8(*S*),9(*R*)-EET. This selectivity for the biologically relevant EET enantiomers suggests that the cEH may play a functional role in the *in vivo* catalysis of EET hydration and in the control of their steady state concentrations.

We describe here the regio- and stereoselective hydration of EETs by rabbit liver and lung cytosol and by a purified cEH preparation. In addition, we demonstrate that, for 8,9- and 14,15-EET, enzymatic oxirane hydration is regioselective with nucleophilic water addition at C_9 and C_{15}. In conclusion, our data suggest that (i) the cEH possesses a highly structured active site that provides for regioselective water addition, (ii) there is an enzymatic pathway for the generation of chiral DHETs, (iii) the catalysis of EET hydration may be one of the endogenous roles for this well characterized enzyme, and (iv) that the cEH may therefore play a key functional role in the *in vivo* metabolism of these potentially important compounds.

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