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Epoxide hydrolases: biochemistry and molecular biology

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Abstract

Epoxides are organic three-membered oxygen compounds that arise from oxidative metabolism of endogenous, as well as xenobiotic compounds via chemical and enzymatic oxidation processes, including the cytochrome P450 monooxygenase system. The resultant epoxides are typically unstable in aqueous environments and chemically reactive. In the case of xenobiotics and certain endogenous substances, epoxide intermediates have been implicated as ultimate mutagenic and carcinogenic initiators Adams et al. (Chem. Biol. Interact. 95 (1995) 57–77) Guengrich (Properties and Metabolic roles 4 (1982) 5–30) Sayer et al. (J. Biol. Chem. 260 (1985) 1630–1640). Therefore, it is of vital importance for the biological organism to regulate levels of these reactive species. The epoxide hydrolases (E.C. 3.3.2.3) belong to a sub-category of a broad group of hydrolytic enzymes that include esterases, proteases, dehalogenases, and lipases Beetham et al. (DNA Cell Biol. 14 (1995) 61–71). In particular, the epoxide hydrolases are a class of proteins that catalyze the hydration of chemically reactive epoxides to their corresponding dihydrodiol products. Simple epoxides are hydrated to their corresponding vicinal dihydrodiols, and arene oxides to *trans*-dihydrodiols. In general, this hydration leads to more stable and less reactive intermediates, however exceptions do exist. In mammalian species, there are at least five epoxide hydrolase forms, microsomal cholesterol 5,6-oxide hydrolase, hepoxilin A_3 hydrolase, leukotriene A_4 hydrolase, soluble, and microsomal epoxide hydrolase. Each of these enzymes is distinct chemically and immunologically. Table 1 illustrates some general properties for each of these classes of hydrolases. Fig. 1 provides an overview of selected model substrates for each class of epoxide hydrolase.

Keywords: Microsomal epoxide hydrolase; Soluble epoxide hydrolase; Cholesterol hydrolase; Hepoxilin A_3 hydrolase; Leukotriene hydrolase

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1. Cholesterol 5,6-oxide hydrolase

1.1. Introduction

The hydration of cholesterol 5,6 α- and 5,6 β-oxide (Fig. 1) to cholestane 3 β, 5 α, 6 β-triol is catalyzed by cholesterol oxide hydrolase, a microsomal hydrolase found in mammalian liver tissues. The gene or cDNA encoding this protein has yet to be cloned or characterized, but is distinct from oxidosqualene cyclase [1]. Cholesterol epoxide hydrolase displays a wide tissue distribution with all tissues tested showing cholesterol epoxide hydrolase activity [2]. Of the tissues tested, liver microsomes have been shown to have the greatest enzymatic activities, five-fold greater than other tissues [2]. In rodents, cholesterol 5,6-oxide hydrolase is inducible by the fibrate drugs such as clofibrate and ciprofibrate [3]. The mechanism of this induction is not understood, but presumably, involves interaction with the peroxisome proliferator-activated receptor α.

1.2. Substrates and inhibitors

As indicated previously, cholesterol oxide hydrolase is a distinct enzyme from oxidosqualene cyclase, as well as from microsomal epoxide hydrolase, the latter being responsible for the metabolism of a wide range of xenobiotic alkene and arene oxides [4,1,5]. Although the cholesterol 5,6 β-oxide is more reactive than the α-oxide upon acid-catalyzed hydration, the α-oxide is a 4.5-fold better substrate than the β-oxide as indicated by values of V_{max}/K_m [1]. The enzyme activity of both 5,6 α- and 5,6 β-oxide diastereomers is product-inhibited by cholestanetriol through a competitive mechanism with the apparent K_i for cholestanetriol being 10.8 and 6.8 μM against cholesterol α- and β-epoxides, respectively [6]. This inhibitory effect of cholestanetriol may account for the difference observed in the hydration rates for the cholesterol epoxide isomers when they are incubated together in the presence of liver microsomes. The parent cholesterol epoxides have been reported to act as weak direct-acting mutagens [7,8]. Although the cholestane-3 β, 5 α, 6 β-triol metabolite was more toxic and a more potent inhibitor of DNA synthesis than the epoxide, it was not significantly mutagenic [7]. Therefore, conditions where conversion rates of the epoxide to the triol are low, as in the case of low-cholesterol epoxide hydrolase activity, appear to favor mutagenicity, whereas rapid conversion to the triol favors cytotoxicity [7].

The rat liver microsomal cholesterol oxide hydrolase is modified and inactivated covalently by the 7-dehydrocholesterol 5,6 β-oxide [9]. Several imino compounds are competitive inhibitors for the enzyme from rat liver. The most effective of these is 5,6 α-iminocholestanol ($K_i = 0.085$ μM) [1]. Inhibition by aziridines is consistent with the participation of acid catalysis in the mechanism of action of the enzyme [1]. Other inhibitors that have been characterized including the inhibitors 7-ketocholesterol, 6-ketocholestanol, and 7-ketocholestanol, with the latter displaying an apparent K_i lower than the K_m for either cholesterol epoxide isomer. None of the xenobiotic epoxide hydrolase inhibitors or activators studied appears to effect cholesterol-epoxide hydrolase activity [6,10].

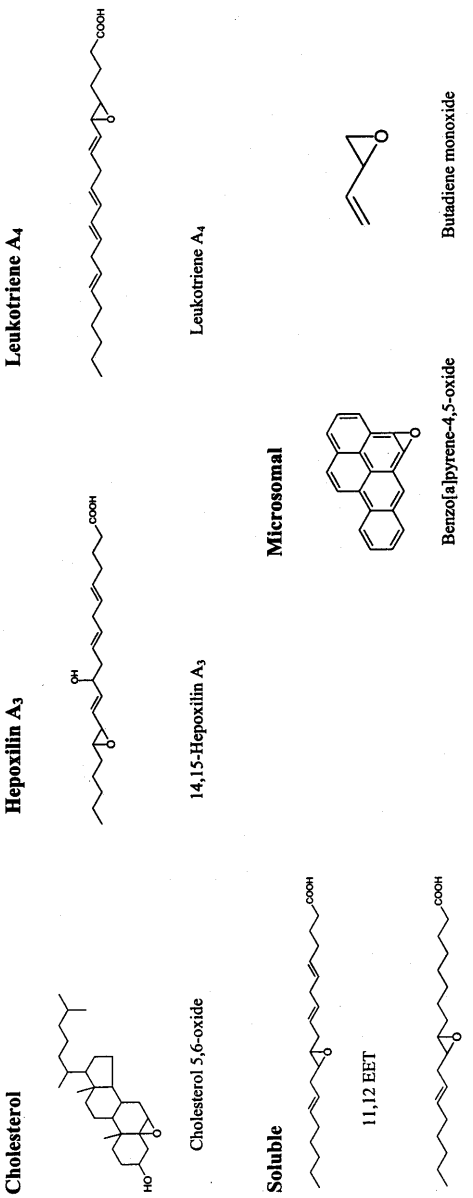

Fig. 1. Model substrates for epoxide hydrolases.

The oxidation of cholesterol proceeds as part of the lipid peroxidation process in membranes. Common oxidation products of lipid peroxide-dependent propagation reactions include the enantiomeric 5,6-epoxides and 7-ketocholestanol [8]. Several of these oxysterol products exhibit cytotoxity, with the cholesterol epoxides demonstrating weakly mutagenic activity [7,8]. Therefore, conversion of cholesterol epoxides to cholestane triol is likely a detoxification function of the hydrolase.

1.3. Mechanism of action/enzymatic mechanism

The rat liver microsomal enzyme cholesterol oxide hydrolase is modified and inactivated covalently by the 7-dehydrocholesterol 5,6 β-oxide, a modification that is likely to occur at the active site of the enzyme since 5,6 α-iminocholestanol, a potent competitive inhibitor of the enzyme, blocks incorporation of 3-[^{3}H]-7-dehydrocholesterol 5,6 β-oxide into the protein [9]. Unlike xenobiotic microsomal epoxide hydrolase, which is not inactivated or inhibited by 7-dehydrocholesterol 5,6 β-oxide, cholesterol oxide hydrolase appears to hydrolyze cholesterol oxides via a positively charged transition state [9]. The mammalian soluble and microsomal epoxide hydrolases have been proposed to belong to the family of α/β-hydrolase-fold enzymes, enzymes that hydrolyze their substrates by a catalytic triad, with the first step of the enzymatic reaction being the formation of a covalent enzyme–substrate ester [5]. Although an enzyme-substrate–ester intermediate is detected in the course of epoxide hydrolysis by microsomal and soluble epoxide hydrolases, no such covalent intermediate between cholesterol epoxide hydrolase and its substrate is detected, indicating that the cholesterol epoxide hydrolase does not, apparently, act by a similar mechanism and is probably not related structurally to microsomal and soluble epoxide hydrolases [5].

2. Hepoxilin A_3 hydrolase

2.1. Introduction

Hepoxilin A_3 hydrolase is a cytosolic enzyme that participates in the metabolism of the arachidonic acid metabolites. Like cholesterol 5,6-oxide hydrolase, the gene and/or cDNA for this enzyme have not been cloned or characterized, therefore, knowledge of this epoxide hydrolase is limited to biochemical analyses. The protein responsible for hepoxilin A_3 epoxide hydrolase activity has been purified and characterized [11]. Tissue screening indicates that this protein is expressed ubiquitously in mammalian tissues, although its biological roles have been studied principally in tissues of vascular or CNS origin [12–14].

2.2. Substrates and inhibitors

Hepoxilin epoxide hydrolase activity was first demonstrated in rat liver cytosol using as substrate hepoxilin A_3, a hydroxy-epoxide derivative of arachidonic acid [11]. Hepoxilin A_3 (Fig. 1) is the preferred substrate for the enzyme, converting it to the trihydroxy metabolite trioxilin A_3. The enzyme is active only marginally toward other epoxides such as leukotriene A_4 and styrene oxide. The M_r, p*I*, and substrate specificity of hepoxilin epoxide hydrolase indicates that this enzyme is distinct from leukotriene A_4 hydrolase [11].

Homogenates of the rat aorta metabolize hepoxilin A_3 via two competing pathways; one involves hepoxilin epoxide hydrolase to form trioxilin A_3, and a second pathway involves conjugation of hepoxilin A_3 with glutathione via glutathione *S*-transferase to form a glutathione conjugate, hepoxilin A_3 [14]. The hepoxilin metabolites likely alter the intracellular concentrations of ions, including calcium and potassium ions, as well dictating alterations in second messenger systems [12]. Recent evidence suggests that the biological actions of the hepoxilins may be receptor-mediated as indicated from data showing the existence of hepoxilin-specific binding proteins in the human neutrophil [15]. Hepoxilin A_3 has also been implicated as an endogenous lipid mediator opposing hypotonic swelling of intact human platelets [16]. Trichloropropene oxide (TCPO) has been reported to inhibit effectively the hepoxilin epoxide hydrolase [14,16].

2.3. Mechanism of action/enzymatic mechanism

The catalytic mechanisms for hepoxilin hydrolase are largely unknown or unreported. Clearly, this is an area that will require additional study to enable more detailed comparisons of enzymatic mechanism among the various epoxide hydrolases.

3. Leukotriene A_4 hydrolase

3.1. Introduction

Leukotrienes are important arachidonic acid-derived metabolites that mediate inflammatory and allergic response. Leukotriene A_4 hydrolase is a bifunctional Zn^{2+} containing enzyme that exhibits both hydrolase activity, as well as aminopeptidase activity [17]. The human gene for leukotriene A_4 hydrolase has been localized to chromosome 12q22, cloned, and characterized [18]. The gene contains 19 exons, spans more than 35 kb, and encodes a protein with an apparent molecular mass of 54 kDa [18]. There have been no reports of genetic variation in leukotriene A_4 hydrolase, although there is evidence for alternative mRNA splicing [19]. The alternately spliced form leads to a truncated protein with an altered molecular mass. However, the functional significance of the splice variant in vivo is unknown at this time. The putative promoter region has been cloned and sequenced, as well

[18]. It contains two xenobiotic response elements (XRE), a phorbol ester response element (AP2), but no definitive TATA box. Upstream of these elements is a consensus sequence for an *Alu* repeat, which also may regulate gene activity [20]. Leukotriene A_4 hydrolase is localized to the cytosolic compartment and its tissue specific expression is ubiquitous [21–23]. The structure, function, and significance of leukotriene A_4 hydrolase were reviewed recently [24].

3.2. Substrates and inhibitors

Human erythrocytes were shown initially to contain a soluble cytosolic epoxide hydrolase for stereospecific enzymatic hydration of leukotriene A_4 (Fig. 1) into leukotriene B_4 [22]. The purified enzyme preparation exhibited a molecular weight of $54\,000 \pm 1000$, an isoelectric point 4.9 ± 0.2, an apparent K_m from 7 to 36 μM for enzymatic hydration of leukotriene A_4, and a pH optimum ranging from 7 to 8. The enzyme was partially inactivated by its initial exposure to leukotriene A_4 and catalyzed slow but detectable enzymatic hydration (pmol/min/mg) of certain arachidonic acid epoxides including (+/−)-14,15-oxido-5,8–11-eicosatrienoic acid and (+/−)-11,12-oxido-5,8,14-eicosatrienoic acid, but not others, including 5,6-oxido-8,11,14-eicosatrienoic acid. Human erythrocyte leukotriene A_4 hydrolase does not catalyze the hydration of either styrene oxide or *trans*-stilbene oxide [22].

Leukotriene A_4 hydrolase is a bifunctional metalloenzyme and contains 1 mol of zinc per mole of protein [24]. The zinc cofactor is necessary for both the enzyme's peptidase and epoxide hydrolase activities. However, at concentrations of zinc $>$ 1:1 molar ratio (metal:enzyme), zinc is a an inhibitor with IC_{50} values of 10 μM for the hydrolase activity, i.e. the conversion of leukotriene A_4 to leukotriene B_4, and 0.1 μM for the peptidase activity [25]. The inhibition of both enzyme activities is reversed by additions of chelating agents such as ethylene diamine tetraacetic acid (EDTA) or dipicolinic acid. Several other divalent cations, in particular $CdSO_4$ and $HgCl_2$, also function to inhibit the hydrolase [25]. The enzyme's peptidase activity is inhibited by $CdSO_4$, $NiSO_4$, $HgCl_2$, $MnCl_2$, $CoCl_2$, and $PbNO_3$, listed in decreasing order of potency (IC_{50} 0.5–10 M) [25]. In a systematic study on the enzyme specificity and the inhibition of its amidase activity with more than 30 synthetic inhibitors, the identification of specific-keto-β-animo ester and thioamine inhibitors, as tight-binding, competitive type transition-state analog inhibitors of the aminopeptidase activity, with K_i values of 46 and 18 nM, respectively [26,27]. Both compounds inhibited also the epoxide hydrolase activity, with the IC_{50} values of 1 and 0.1 μM for 26 and 27, respectively [26]. Since the enzymatic product, leukotriene B_4, is an inflammatory mediator, and leukotriene A_4 hydrolase is distributed widely in various cell types and tissues, this activity likely contributes important biological function [28,24].

3.3. Mechanism of action/enzymatic mechanism

From sequence comparisons with aminopeptidases, a tyrosine at position 383 in leukotriene A_4 hydrolase identified as a possible catalytic residue and tested in this

respect using site-specific mutagenesis to substitute this position with phenylalanine, histidine or glutamine [29]. The mutated proteins exhibited peptidase activities $< 0.3\%$ that of the wild-type enzyme. However, the epoxide hydrolase function was not impacted to the same degree, corresponding to 11, 16 and 17% that of the wild-type enzyme for the Tyr^{383}Phe, Tyr^{383}His and Tyr^{383}Glu leukotriene A_4 hydrolases, respectively. The results from this study are consistent with a role for Tyr^{383} in the peptidase reaction of leukotriene A_4 hydrolase, where it may act as a proton donor in a general base mechanism. These and other data provide evidence that the two catalytic activities of leukotriene A_4 hydrolase involve non-identical but overlapping active sites [29,30]. Evidence for a carbocation intermediate has been reported in the enzymatic transformation of leukotriene A_4 into leukotriene B_4 [31]. Based on sequence alignment studies, leukotriene A_4 hydrolase is likely not related to the other epoxide hydrolases, in particular soluble and microsomal epoxide hydrolase, and does not share a common enzymatic mechanism [32].

4. Soluble epoxide hydrolase

4.1. Introduction

Soluble epoxide hydrolase (or cytosolic epoxide hydrolase) is a xenobiotic metabolizing enzyme that also participates in the metabolism of endogenously derived fatty acid epoxides [33]. The protein responsible for soluble epoxide hydrolase (sEH) activity has been purified and characterized [34–37]. sEH activity is apparently expressed in all tissues [38]. Purified sEH protein from mammalian species possesses an imperfect peroxisomal targeting sequencing aT at the C-terminus [36,37,39]. Although the majority of the enzymatic activity is observed in the cytosolic fraction, activity in the peroxisomal fraction also has been detected [38–40]. The gene and/or cDNAs of sEH from a variety of different species have been cloned and characterized [36,41–44]. The human gene is localized to chromosome 8p21-p12, spans approximately 45 kb, and consists of 19 exons, with the first exon being non-coding [43].

Variation in sEH has been observed in human populations. Past investigations have demonstrated an 11-fold variation in sEH activity in unstimulated lymphocytes [45]. Results of twin and family studies examining sEH activities suggest predominantly genetic control with either monogenic or polygenic control mechanisms [45]. Further studies have indicated the presence of genetic polymorphism at the sEH gene locus [46]. Rodent species exhibit an induction of sEH activity when treated with peroxisomal proliferator agents, such as 2,4-dichlorophenoyacetic acid and Wyeth 14.643, and hypolipidemic drugs, such as clofibrate [47–51]. The mechanism of this induction has not been investigated, but involves likely the peroxisomal proliferator-activated receptor α pathway. There has been no reported induction of sEH in human populations.

4.2. Substrates and inhibitors

sEH in general catalyzes the hydrolysis of *trans*-substituted epoxides, as well as various aliphatic epoxides derived from fatty acid metabolism [33,52]. The prototypic substrate used to distinguish sEH activity from microsomal epoxide hydrolase activity is *trans*-stilbene oxide (Fig. 1) [51]. Other more sensitive radiometric substrates have been developed for sEH [53]. These include [^{14}C]*cis*-9,10-epoxystearic acid and [2-^{3}H]*trans*-1,3-diphenyl-propene oxide. The latter substrates provide a higher sensitivity with increased maximal velocities when compared with *trans*-stilbene oxide. Several additional specific sEH substrates have been developed and characterized [54], including a series of epoxy esters and carbonate-derivatives that cyclize spontaneously upon hydrolysis of the epoxide, releasing an alcohol. These substrates, therefore, enable rapid spectrophotometric analysis of sEH activity without the use of radiolabeled probes. Numerous investigations have demonstrated the importance of sEH in the metabolism of diepoxy fatty methylesters and epoxyeicosatrienoic acids (EETs) [52,55,56]. Recently, sEH was shown to bioactivate leukotoxin (Fig. 1), a linoleic acid metabolite [57]. These latter substances represent endogenous metabolic products of fatty acid metabolism and implicate a potentially important role for sEH in mediating pathophysiologic and physiologic processes.

Several classes of sEH inhibitors have been developed. Early work characterized chalcone oxide derivatives as inhibitors of sEH [58]. Later studies characterized *trans*-3-phenylglycidols as inhibitors of rodent, monkey and human sEH, but not microsomal epoxide hydrolase [59,60]. More recently, potent competitive inhibitors of sEH based on urea and carbamates have been characterized. The most potent of these inhibitors have K_Is in the nanomolar range [61]. The heavy metals Cd^{2+} and Cu^{2+} have been identified as competitive inhibitors of sEH, and may represent a mechanism of sEH regulation during inflammation [62].

The importance of sEH in normal metabolism of endogenous metabolites of fatty acids is well established. Leukotoxin is bioactivated via sEH metabolism [57]. Leukotoxin is an oxidative metabolite of linoleic acid in leukocytes, that has been associated with multiple organ failure and adult respiratory distress syndrome seen in some severe burn patients. Epoxyeicosatrienoic acids (EETs) are P450 derived metabolites of arachidonic acid that possess a multitude of biologic activities. These include activation of Ca^{2+}-activated K^+ (K_{ca}) channels inducing a potent relaxation of blood vessels, thereby implicating EETs as candidates for endothelium-derived hyperpolarization factors (EDHF) in coronary blood vessels [63,64]. sEH has been shown to regulate EET incorporation into phospholipids [65], suggestive of sEH modulation of endothelial function via activation of K_{ca} channels.

4.3. Mechanism of action/enzymatic mechanism

Of the known epoxide hydrolases, sEH is associated likely with the most in depth characterization with respect to enzymatic mechanism and structure. This information has been forthcoming from numerous sequence comparisons, biochemical, and

crystallographic investigations. Based on sequence alignment analyses, it was concluded that sEH belongs to a broad group of α/β hydrolase enzymes that include bacterial haloalkane dehalogenase [32,66]. The alignment characterization also lead to identification of a proposed catalytic triad of amino acids within sEH. Mutagenesis experiments implicated Asp^{333} and His^{523} in the catalytic mechanism for sEH, similar to other α/β hydrolase enzymes [67]. The results of the latter investigation indicated that since His^{523} was conserved in all epoxide hydrolase across species, and mutation of His^{523} in murine sEH resulted in activity of 0.1% of wild-type activity, this residue was likely a key participant in the catalytic mechanism for the hydrolase. Further biochemical analyses revealed the formation of an α-hydroxyacyl-enzyme intermediate initiated by amino acid Asp^{333} [68]. This intermediate was hydrolyzed by the addition of water to the carbonyl carbon of the ester bond, yielding the dihydrodiol product. Other data also supported the role of Asp^{333} and His^{523} in the catalytic mechanism, and implicated further the participation of the Asp^{495} residue [69]. Results from the latter study, along with others, suggest a nucleophilic attack of the oxirane ring by Asp^{333} leading to the formation of the α-hydroxyacyl-enzyme intermediate shown by Borhan et al. [68]. The His^{523}–Asp^{495} pair creates an environment that abstracts a proton from a water molecule, thereby activating it and hydrolyzing the α-hydroxyacyl-enzyme intermediate, yielding the dihydrodiol product. The crystal structure of recombinant murine sEH was elucidated in 1999 by Argiriadi et al. [70]. The results of the crystal data confirmed previous enzymological investigations that led to the identification of the catalytic triad of sEH. Interestingly, the crystal analysis demonstrated a vestigial active site in the N-terminal domain of sEH and its similarity to the active site in bacterial haloacid dehalogenase. In sEH, this vestigial site does not participate in epoxide hydrolysis, but does play an important role in the stabilization of the dimeric structure of sEH.

5. Microsomal epoxide hydrolase

5.1. Introduction

Of the known epoxide hydrolases, microsomal epoxide hydrolase (mEH) was the first characterized. mEH catalyzes the *trans*-addition of water to a broad range of epoxide substrates. There has been great interest in mEH because of its potential involvement in the bioactivation of carcinogenic polycyclic aromatic hydrocarbons [71]. However, mEH does play an important role in the detoxification of many reactive epoxide intermediates [72,73]. There appears to be universal expression of mEH in all tissues studied to date [72,74]. The highly conserved protein catalyzing mEH activity has been purified and the protein sequence determined in several mammalian species [32,75–78]. The gene and cDNA for mEH has been cloned, sequenced in humans [77,79]. The gene exists as a single copy in the haploid genome and has been localized to the long arm of chromosome 1 [77]. The gene encompasses approximately 20 kb and is comprised of eight coding exons, one non-coding

exon, and encodes for a single monomeric protein of 455 amino acids. The 5′-flanking region of mEH has been cloned and characterized [80], and human mEH expression levels appear to be effected only modestly by exposures to common chemical inducers in primary hepatocyte culture [81]. However, in rodent species, mEH appears to be highly inducible by a number of prototypic chemicals [82–85]. In vitro transfection studies using promoter constructs have indicated a repression of epoxide hydrolase driven chloramphenicol *O*-acetyltransferase (CAT) activity and epoxide hydrolase mRNA by dexamethasone [86].

There exist certain well-characterized genetic polymorphisms in human mEH [87,88]. The coding region of mEH presents with two prominent genetic polymorphisms. The first, within exon 3, results in the substitution of Tyr with His at amino acid position 113; another, within exon 4, codes for the substitution of a His with Arg at amino acid position 139. In transiently transfected cells, using benzo[a]pyrene-4,5-oxide as substrate probe, the Tyr^{113}/Arg^{139} variant exhibited approximately 2-fold higher levels of enzymatic activity than the His^{113}/His^{139} variant when normalized to total S9 protein or mEH mRNA of the transfected cell extracts [88]. However, when results were normalized to mEH immunodetectable protein levels, the variants appeared to encode only marginal phenotypic differences. These data, together with results from additional studies [89,90], suggest that differences in protein stability among the variant mEH proteins may factor into the basis of observed interindividual differences in mEH phenotype. Additionally, seven other 5′-flanking region genetic polymorphisms have been described [91], and may also impact mEH gene transcriptional activity and resulting cellular phenotype.

5.2. Substrates and inhibitors

In contrast to sEH and other epoxide hydrolases, the major role of mEH is likely oriented toward the metabolism of xenobiotic compounds. The substrates of mEH are generally quite specific to this hydrolase, with little or no activity exhibited towards the other epoxide hydrolases. Typical substrates include toxic and procarcinogenic compounds, as well as commonly used anticonvulsant drugs. The most common environmental compound metabolized by mEH are epoxide derivatives of polycyclic aromatic hydrocarbons (Table 1, benzo[a]pyrene 4,5-oxide). The dihydrodiol products of mEH metabolism have been implicated as ultimate reactive carcinogens that are responsible for polycyclic aromatic hydrocarbon-initiated carcinogenesis. Other xenobiotic compounds of note that are metabolized by mEH are the epoxide derivatives of 1,3-butadiene (Table 1) [92–94], benzene [95,96], aflatoxin B_1 [97–99], chrysene [100,101], nitropyrene [102], naphthalene, and anthracene [103,104]. The importance of mEH is underscored by data obtained from a mEH knockout mouse model that show reduced carcinogenicity of 7,12-dimethylbenz[a]anthracene in knockout versus control animals [105]. The most commonly used probe substrates for mEH activity are *cis*-stilbene oxide [106–108] and styrene oxide [109–111]. The former is commonly used to differentiate between microsomal and soluble epoxide hydrolase activity. Important clinical drugs metabolized by mEH include epoxide derivatives of anticonvulsant drugs, phenytoin [112–114] and

Table 1
Characteristics of mammalian epoxide hydrolases

Enzyme	Localization	Substrates		Inhibitors
		Endogenous	Xenobiotic	
Cholesterol 5,6-oxide	Microsomal	α- and β-Cholesterol 5,6 epoxide	Unknown	Cholestanetriol, 7-dehydrocholsterol 5,6 β-oxide
Hepoxilin A_3	Cytosolic	Arachidonic acid epoxides	Unknown	Trichloropropene oxide
Leukotriene A_4	Cytosolic	Leukotriene A_4	Unknown	Divalent cations, α-keto-β-amino esters
Soluble	Primarily cytosolic, secondarily peroxisomal	EETs, leukotoxin	*Trans*-stilbene oxide	Chalcone oxides, urea and carbamate derivatives, Cd^{2+}, and Cu^{2+}
Microsomal	Microsomal	Steroid epoxides, androstene epoxide, estroxide	PAH epoxides, phenytoin, carbamazepine	1,1,1-Trichloropropene-2,3-oxide, divalent heavy metals, cyclopropyl oxiranes

carbamazepine [115,116]. mEH has been hypothesized to play a role in the endogenous metabolism of estroxide and androstene oxide [117].

Inhibitors of microsomal epoxide hydrolase have been developed and characterized. Early studies identified 1,1,1-trichloropropene-2,3-oxide as an inhibitor of both rodent and human mEH [118]. Later studies demonstrated that the cyclopropyl oxiranes were competitive reversible inhibitors of mEH, with some having equal IC_{50} of 1,1,1-trichloropropene-2,3-oxide [119]. Recently, divalent heavy metals were shown to inhibit mEH enzymatic activity [62]. The most potent of the inhibitors was Zn^{2+} and Hg^{2+} with IC_{50}s of approximately 20 μM.

mEH plays an important role in the metabolism of many xenobiotic compounds, including common toxicants and clinical drugs. Common anticonvulsant drugs (i.e. phenytoin, carbamazepine, etc.) are associated with a potentially lethal hypersensitivity syndrome, with symptoms that include fever, rash, lymphadenopathy, and hepatitis [120]. Since mEH does metabolize these compounds and is known to be polymorphic in human populations, it may be responsible for the idiosyncratic hypersensitiviy syndrome. In fact, Shear et al. have reported an association of an inherited defect in microsomal epoxide hydrolase activity with drug toxicity [121]. However, more recent investigations have not confirmed these associations [87,122]. Other predisposing factors in these reactions may relate to high levels of circulating autoantibodies to P450, suggesting an immunologic disease mechanism [123]. Polymorphisms in mEH too may lead to differences in host-mediated bioactivation of procarcinogens resulting in differential susceptibility to cancers of various tissues. Numerous human epidemiological studies have examined the role of the polymorphism in mEH and tumor formation [124–128]. These data suggest that mEH genotype is associated with altered risk of several types of cancers and pulmonary disease.

5.3. Mechanism of action/enzymatic mechanism

Although a crystal structure for mEH has not been produced, there is a good understanding of its enzymatic mechanism. Like sEH, mEH is a member of the α/β-hydrolase enzyme family [66]. Based on its similarity with bacterial haloalkane dehalogenases and other epoxide hydrolases, a catalytic triad consisting of a His^{431}, Asp^{226}, and a Glu^{404} has been proposed [129,130]. These findings are based on earlier reports of a His at position 431 that is essential for catalysis [131], and sequence alignments of related epoxide hydrolases. The mechanism of mEH generally follows a mechanism similar to that of sEH, with the attack of the oxirane ring to yield an alkyl–enzyme intermediate followed by subsequent hydrolysis of the intermediate by water [132]. An interesting finding is the similarity of sEH from *Aspergillus niger* (*A. niger*) to mammalian mEH [133]. The protein from this species shares a similar catalytic triad to mammalian species and is also a member of the α/β-hydrolase enzymes. Recently, the crystal structure for epoxide hydrolase from *A. niger* was elucidated [134]. The findings of this report indicate that due to the narrow hydrophobic tunnel of the active site, mEH is unable to catalyze the hydrolysis of bulky *trans*-substituted epoxides. It also suggests a role for residues

320–328 in the interaction of mEH with other proteins, possibly epoxide-generating proteins such as the cytochrome P450s.

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