Biochemical and Biophysical Research Communications 419 (2012) 796–800

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Role of soluble epoxide hydrolase phosphatase activity in the metabolism of lysophosphatidic acids

Christophe Morisseau *, Nils Helge Schebb [1], Hua Dong, Arzu Ulu, Pavel A. Aronov [2], Bruce D. Hammock

Department of Entomology and UCD Cancer Center, One Shields Avenue, University of California, Davis, CA 95616, USA

ARTICLE INFO

Article history:
Received 31 January 2012
Available online 24 February 2012

Keywords:
Soluble epoxide hydrolase
Phosphatase
Lysophosphatidic acid

ABSTRACT

The EPXH2 gene encodes for the soluble epoxide hydrolase (sEH), which has two distinct enzyme activities: epoxide hydrolase (Cterm-EH) and phosphatase (Nterm-phos). The Cterm-EH is involved in the metabolism of epoxides from arachidonic acid and other unsaturated fatty acids, endogenous chemical mediators that play important roles in blood pressure regulation, cell growth, inflammation and pain. While recent findings suggested complementary biological roles for Nterm-phos, its mode of action is not well understood. Herein, we demonstrate that lysophosphatidic acids are excellent substrates for Nterm-phos. We also showed that sEH phosphatase activity represents a significant (20–60%) part of LPA cellular hydrolysis, especially in the cytosol. This possible role of sEH on LPA hydrolysis could explain some of the biology previously associated with the Nterm-phos. These findings also underline possible cellular mechanisms by which both activities of sEH (EH and phosphatase) may have complementary or opposite roles.

1. Introduction

The EPHX2 gene encodes for the soluble epoxide hydrolase (sEH), a cytosolic ubiquitous enzyme in mammals [1]. The sEH is expressed in many tissues including liver and kidneys, but also vascular endothelium, leukocytes, red blood cells, smooth muscle cells, adipocytes and the proximal tubule [1,2]. The sEH protein is a homodimer with a monomeric unit of 62.5 kDa [3], which has two distinct activities in two separate structural domains of each monomer: the C-terminal epoxide hydrolase activity (Cterm-EH; E.C. 3.3.2.10) and the N-terminal phosphatase activity (Nterm-phos; E.C. 3.1.3.76) [3]. The C-terminal is the site of the epoxy-fatty acid hydrolysis that is responsible for the biology associated with EPHX2 [1,4–6], while a magnesium dependent hydrolysis of phosphate esters was recently associated with the N-terminal domain of sEH [7]. The Cterm-EH hydrolyzes epoxy-eicosatrienoic acids (EETs) and other epoxy-fatty acids [8], which are potent endogenous signaling molecules [1,4]. Pharmacological inhibition of Cterm-EH by potent selective inhibitors [9], has resulted in anti-inflammatory [4,5], anti-hypertensive [10], neuro-protective [11], and cardioprotective [4] effects in animal models. Furthermore, Cterm-EH inhibition resulted in beneficial effects in more complex diseases such as diabetes and metabolic syndrome [12,13].

There are several lines of evidence indicating a biological role for the sEH phosphatase activity (Nterm-phos). The sEH-null mice that lack both Cterm-EH and Nterm-phos activities have lower cholesterol and steroid levels [14]. Furthermore, in recombinant Hep G2 cells, Cterm-EH activity lowered cholesterol synthesis while Nterm-phos activity increased it [15]. Put together, this suggests that sEH regulates cholesterol levels *in vivo* and *in vitro*, and that the Nterm-phos is a potential therapeutic target in hypercholesterolemia-related disorders. Similarly, in recombinant endothelial cells, both Cterm-EH and Nterm-phos activities contribute to growth factor expression and cell growth [16]. In mice, it seems that the Nterm-phos may play a role in the development of hypoxia-induced pulmonary hypertension [17]. The phosphatase activity of sEH has been shown recently to play a pivotal role in the regulation of eNOS activity and NO-mediated endothelial cell functions [18]. Human polymorphism studies have shown that the Arg287Gln polymorph of sEH is associated with the onset on coronary artery calcification in African–American subjects [19], and insulin resistance in type 2 diabetic patients [20]. This SNP (G860A) of sEH reduces both Cterm-EH and Nterm-phos activities

Abbreviations: CMC, critical micelle concentration; LC–ESI-MS, liquid chromatography electrospray ionization mass spectrometry; LPA, lysophosphatidic acid; MAG, mono-acyl-glycerol; sEH, soluble epoxide hydrolase; *t*-DPPO, *trans*-diphenylpropene oxide.

* Corresponding author. Fax: +1 530 751 1537.
E-mail address: chmorisseau@ucdavis.edu (C. Morisseau).
[1] Current address: Institute for Food Toxicology and Analytical Chemistry, University of Veterinary Medicine Hannover, Bischofsholer Damm 15, 30173 Hannover, Germany.
[2] Current address: Stanford University, Mass Spectrometry, 333 Campus Dr., Mudd. 175, Stanford, CA 94305, USA.

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2012.02.108

[21,22]. Furthermore, people having a Lys55Arg polymorph of sEH, which has reduced Nterm-phos but increase Cterm-EH, have higher risk of coronary heart diseases. This SNP also increases the long-term risk of ischemic stroke in men [23].

The biological role of an enzyme is intimately linked to the natural substrate(s) it transforms and/or to the products made. Nterm-phos activity was first described to hydrolyze dihydroxy lipid phosphates [7]. Unfortunately, following development of a sensitive LC–MS-MS analytical method, this class of lipid phosphate was never identified in biological fluids and tissue extracts. More recently, terpenic pyrophosphates, which are cholesterol precursors, were shown *in vitro* to be substrates for Nterm-phos [24,25], supporting the hypothesis that Nterm-phos is a lipid phosphate phosphatase. However, the observation that Nterm-phos activity increased cholesterol synthesis in cell cultures [15] suggests that terpenic pyrophosphates are not hydrolyzed *in vivo* by Nterm-phos. Furthermore, hydrolysis of such substrates does not explain the effect of Nterm-phos on cell growth [16]. Recently, Nterm-phos was shown to modulate endothelial cell functions by altering the phosphorylation of endothelial nitric oxide synthase (eNOS) [18]. While no direct proof was presented, this observation lets one hypothesize that Nterm-phos is a protein phosphatase. To distinguish among multiple hypotheses, we screened 30 natural phosphate containing chemicals as Nterm-phos substrates, and assess the role of sEH phosphatase activity in the metabolism of those key compounds in tissue extracts.

2. Materials and methods

2.1. Chemicals

Compounds **1**–**12** were from Sigma Aldrich, **13**–**15** from Anaspec (San Jose, CA), compounds **16**–**29** were obtained from Avanti Polar Lipids (Alabaster, AL), and compound **30** was provided by Alfa Aesar (Ward Hill, MA). The attophos substrate was obtained from Promega (Madison, WI). All other chemicals and solvents were obtained from Fisher Scientific (Pittsburgh, PA) or Sigma Aldrich (St. Louis, MO), and were of the highest purity available.

2.2. Enzyme preparations

Recombinant human sEH was produced in a baculovirus expression system [26], and purified by affinity chromatography [27]. This enzyme preparation was at least 97% pure, based on SDS–PAGE followed by scanning densitometry. The enzyme preparation was kept at −80 °C until use. Protein concentration was quantified by using the Pierce BCA assay using Fraction V bovine serum albumin as the calibrating standard.

2.3. Screening and inhibition assay

A library of 29 natural phosphate containing compounds was created in black polystyrene 96-well plates. Furthermore, dodecyl-phosphate, a known inhibitor of Nterm-phos [24], was used as positive control. Each compound was dissolved at 0.5 mM in a 80:20 mixture of BisTris/HCl buffer (pH 7.0; 25 mM) and DMSO containing 0.02% of Tween 20, and 1 mM $MgCl_2$. Six wells (three for the blank and three for the control 100% activity) received 20 μL of the solvent mixture. For each compound, 20 μL of the solution at 0.5 mM were dispensed in three wells. The exact composition of the plate is given in Fig. S1. The screening of this plate was performed using attophos as substrate [24]. Simplistically, 150 μL of a 2.8 nM solution of purified human sEH in BisTris/HCl buffer (pH 7.0; 25 mM) containing 1 mM $MgCl_2$ and 0.1 mg/mL of BSA (buffer A) was added to the wells ($[E]_{final}$ = 2.1 nM; [tested compounds]$_{final}$ = 50 μM). To monitor background hydrolysis, the enzyme was replaced by 150 μL of buffer A. After throughout mixing and pre-incubation at room temperature for 0.5, 15, 30, 60 or 90 min, the reaction was started by the addition of 30 μL of a 16.7 μM solution of Attophos in buffer A ($[S]_{final}$ = 2.5 μM). After 10 min incubation at room temperature in the dark, 100 μL of 0.1 M of NaOH in water were added to each well. Following strong mixing, the amount of fluorescent alcohol produced was measured (λ_{ex} 435 nm, λ_{em} 555 nm, λ_{cutoff} 515 nm) with a SpectraMax M2 microplate reader (Molecular Devices, Sunnyvale, CA, USA) at room temperature. The results given are average ± standard deviation from three separate plates.

Inhibition potencies (IC_{50}s) for the Nterm-phos activity were determined using Attophos A ($[S]_{final}$ = 25 μM) as substrate [24]. IC_{50}s for the Cterm-EH activity were determined using racemic cyano(2-methoxynaphthalen-6-yl)methyl *trans*-(3-phenyl-oxyran-2-yl) methyl carbonate ($[S]_{final}$ = 2.5 μM) [28]. For comparison purpose, the assays for both activities were run with the same enzyme concentration ($[E]_{final}$ = 2.0 nM) and in buffer A. The human sEH was incubated with the inhibitors for 5 min in buffer A at 30 °C prior to substrate addition. The activities were measured by following the formation of the fluorescent products for 10 min at 30 °C as described [24]. By definition, IC_{50} is the concentration of inhibitor that reduces enzyme activity by 50%. Reported IC_{50} values are the average ± SD (n = 3).

2.4. Kinetic assay conditions

Kinetic parameters for a series of lysophosphatidic acids and sphingosine phosphates were determined under steady-state conditions using the purified recombinant human sEH. One microliter of substrate solution in water or ethanol ($[S]_{final}$ from 1.0 to 50 μM; 7–8 concentrations used for each substrate) was added to 100 μL of the enzyme solution at 2.6 μg/mL ($[E]$ = 40 nM) in Buffer A. The reaction mixtures were incubated at 30 °C for 5–30 min. The reactions were then quenched by adding 100 μL of a 50:49:1 mixture of acetonitrile, water and acetic acid (a 99:1 mixture of acetonitrile and acetic acid was used for 1-stearoyl-glycero-3-phosphate) containing 200 nM of hexanoyl-ceramide (CER-6) as internal standard. The quantity of alcohol formed was then determined by Liquid chromatography electrospray ionization mass spectrometry (LC–ESI-MS) as described below. The kinetic constants (V_M and K_M) were calculated by non-linear fitting of the Michaelis–Menten equation to the results obtained using Sigma Plot version 11.0 (Systat. Software Inc.; Chicago, IL). Results are means ± SD (n = 3).

2.5. Quantification of sphingosine, ceramides and mono-acyl glycerols (MAG)

A liquid chromatography electrospray ionization mass spectrometry (LC–ESI-MS), which allows the quick quantification of the products was developed based on previously described methods for lipids [29]. A complete description and performance of the method is given in Supplementary data. In brief, separation was carried out on short RP-column (20 × 2.1 mm) yielding in a fast analysis time of less than 2 min per sample. MS detection was carried out after positive electrospray ionization in selected reaction monitoring mode. With dynamic ranges (r^2 > 0.99) from 3 to 10,000 nM for the products (Table S2), the method allowed the quantitatively monitoring of enzyme conversions rates of at least 0.3% of the substrate.

2.6. Tissue preparation

Liver and lungs tissues were collected post-mortem from ≈40 g male Swiss–Webster mice (n = 6). All samples were flash-frozen

with liquid nitrogen, and stored at −80 °C until used. After thawing, samples were then homogenized in chilled sodium phosphate buffer (100 mM, pH 7.4; buffer B) containing 1 mM PMSF, EDTA and DTT. The homogenate was centrifuged for 1 h at 100,000*g* at 4 °C, and the supernatants were collected. The pellets were resuspended in chilled sodium phosphate buffer B and centrifuged for 1 h at 100,000*g* at 4 °C. The second supernatants were discarded, while the resulting pellets were suspended in buffer B. Both supernatants and pellets solutions were aliquot and flash-frozen with liquid nitrogen, and stored at −80 °C until use.

2.7. Activity assay

LPA hydrolysis activity in tissue was measured using compound 16 as substrate. One microliter of a 5 mM solution of 1-myristoyl-glycerol-3-phosphate **16** in water ($[S]_{final}$ = 50 μM) was added to 100 μL of the purified human sEH ([*E*]: 10 nM ≈ 0.7 μg/mL) or of tissue extracts diluted in Buffer A. The reaction mixtures were incubated at 30 °C for 5–30 min. The reactions were then quenched by adding 100 μL of a 50:49:1 mixture of acetonitrile, water and acetic acid containing 200 nM of CER-6 as internal standard. The quantity of alcohol formed was then determined by LC–MS analysis as described above. The Cterm-EH activity in tissues was measured using [^{3}H]-*trans*-diphenylpropene oxide (*t*-DPPO) as substrate [30].

3. Results

3.1. Screening of library of natural phosphates

Table 1 provides the results of Nterm-phos inhibition by a series of natural phosphates. We screened at a concentration of phosphate compounds of 50 μM that is less than the critical micelle concentration for most of the compounds tested [31]. After 30 s incubation, we observed significant Nterm-phos inhibition by lipid phosphates **1**–**3**, **16**–**22**, **27** and **29**, supporting the role of sEH phosphatase activity as a lipid phosphate phosphatase. Looking at effects on the Cterm-EH under the same conditions, we observed significant inhibition only for compounds **21** (20 ± 3%) and **22** (12 ± 2%). While compounds **1**–**3** were shown to be Nterm-phos substrates [24,25], neither **16**–**20** nor **27** and **29** were shown to interact with sEH before. When looking at time dependence of Nterm-phos inhibition by these compounds (Fig. S3), except for **19** and **20**, the inhibition decreased over time for the other compounds, suggesting that they are substrates of sEH phosphatase. For **19** and **20**, we observed increasing inhibition of Nterm-phos with time, suggesting that their hydrolytic products (C18:1 MAG and C20:4 MAG, respectively) could inhibit Nterm-phos. Using attophos as reporting substrate, we observed that the hydrolytic products of **19** and **20** can inhibit Nterm-phos in a dose dependent manner (Fig. S4). Kinetic analysis revealed a non-competitive mechanism with K_I of 30 ± 5 and 5 ± 2 μM for C18:1 MAG and C20:4 MAG, respectively. Because the K_I value for C18:1 MAG is around its CMC [31], and because we observed that such concentrations of C18:1 MAG also reduce by ≈50% Cterm-EH, the action of this compound on sEH is neither potent nor specific. On the contrary, the action of C20:4 MAG seems more specific because its K_I is below its CMC and that such concentrations did not significantly affect Cterm-EH.

3.2. Kinetic constants

In order to confirm that compounds **16**–**20**, **27** and **29** are substrates for sEH phosphatase, we developed an analytical method for their hydrolytic products. Using this method and recombinant purified human sEH, we determined the kinetic constants (K_M and k_{cat}) for these endogenous lipids. Examples of kinetic data obtained are given in Fig. 1. As shown in Table 2, they are all substrates of Nterm-phos. Compared to the previous reported Nterm-phos substrate **2** [24], the lysophosphatidic acids (LPA; **16**–**20**) are all at least an order of magnitude better substrate for Nterm-phos. Interestingly, for **19** and **20** we observed inhibition at high substrate concentration (see Fig. 1 for **19**). For **19**, a better fitting was obtained for a cooperative model (n = 2) than for the simple Michaelis–Menten equation. Such cooperative effects were previously observed with some Nterm-phos inhibitors [24]. Sphingosine-phosphate **27** and the corresponding N-acetyl ceramide phosphate **29** were also substrates, but they were hydrolyzed by Nterm-phos much less efficiently than the LPAs **16**–**20**.

3.3. LPA hydrolysis activity in tissues

In tissues, LPAs hydrolysis has mainly been described being attached to membranes [32]. Because sEH is soluble (mostly cytosolic), one could think that, based on published data, its role in LPA hydrolysis is negligible. To measure such activity, LPAs are classically used at very high dose (millimolar range) with Triton X-100 (1–5 mM) to form micelles and in absence of Mg^{2+} [33]. Interestingly, micelle formation was previously shown to inhibit Cterm-EH activity [1]. Using attophos as reporting substrate, we found that concentrations of Triton X-100 above its CMC of 0.5 mM totally inhibit Nterm-phos. Furthermore, Mg^{2+} is necessary

Table 1
Effect of natural phosphates on Nterm-phos.[a]

#	Name	Inhibition (%)	#	Name	Inhibition (%)
1	Geranyl-pyro-phosphate	30 ± 2*	16	1-Myristoyl-2-hydroxy-3-glycerophosphate	63 ± 1*
2	Farnesyl pyro-phosphate	16 ± 3*	17	1-Palmityl-2-hydroxy-3-glycerophosphate	15 ± 3*
3	Geranyl–geranyl-pyro-phosphate	16 ± 4*	18	1-Stearyl-2-hydroxy-3-glycerophosphate	14 ± 1*
4	Glucose-6-phosphate	9 ± 2	19	1-Oleoyl-2-hydroxy-3-glycerophosphate	24 ± 4*
5	Adenosine triphosphate	0 ± 2	20	1-Arachidonoyl-2-hydroxy-3-glycerophosphate	32 ± 3*
6	Guanosine triphosphate	0 ± 2	21	1,2-Dioleoyl-3-glycerophosphate	21 ± 2*
7	Creatine phosphate	9 ± 5	22	Glycerol 1-oleoyl-2-hydroxy-3-phospho-glycerol	12 ± 3*
8	Phospho-threonine	6 ± 2	23	Glycerol 1-oleoyl-2-hydroxy-3-phospho-ethanolamine	4 ± 8
9	Phospho-serine	8 ± 4	24	Glycerol 1-oleoyl-2-hydroxy-3-phospho-choline	4 ± 4
10	Phospho-tyrosine	9 ± 2	25	Glycerol 1-oleoyl-2-hydroxy-3-phospho-serine	11 ± 6
11	α-Glycerophosphate	6 ± 4	26	Glycerol 1-oleoyl-2-hydroxy-3-phospho-inositol	−1 ± 2
12	β-Glycerophosphate	9 ± 4	27	Shingosine-1-phosphate	16 ± 1*
13	H-Gly-Arg-Pro-Arg-Thr-Ser-phosphoSer-Phe-ala-glu-Gly-OH	4 ± 8	28	*N*-Octyl-ceramine-phosphate	−2 ± 5
14	Ac-Glu-Leu-Glu-Phe-phosphoTyr-Met-Asp-Tyr-Glu-NH_2	7 ± 3	29	*N*-Acetyl-ceramide-phosphate	30 ± 7*
15	H-Leu-Lys-Arg-Ala-phosphoThr-Leu-Gly-OH	9 ± 5	30	1-Dodecyl-phosphate	99 ± 1

[a] Attophos was substrate ([*S*] = 2.5 μM). [*I*] = 50 μM.
* Significantly different from control, *t* test $P < 0.01$.

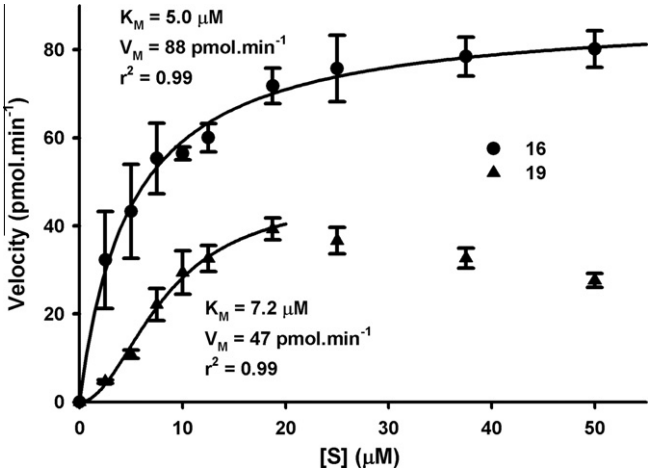

Fig. 1. Determination of the kinetic constants for 1-myristoyl-2-hydroxy-3-glycerophosphate (**16**) and 1-oleoyl-2-hydroxy-3-glycerophosphate (**19**) with the human sEH ($[E]_{final} \approx 40$ nM) in bis-Tris HCl buffer (25 mM, pH 7.0) containing 0.1 mg/mL of lipid free BSA and 1 mM of $MgCl_2$ at 30 °C. The kinetic constants (K_M and V_M) were calculated by non-linear fitting of the Michaelis equation using the enzyme kinetic module of SigmaPlot version 9.01 (Systat. Software Inc., Chicago IL).

Table 2
Kinetic constants of recombinant purified human sEH for lipid phosphates.

#	K_M (μM)	k_{cat} ($10^{-3}.s^{-1}$)	k_{cat}/K_M ($10^{-3}.s^{-1}.\mu M^{-1}$)	n	r^2
2[a]	10 ± 1	14 ± 1	1.3 ± 0.2	1	
16	5.1 ± 1.6	354 ± 10	76 ± 21	1	0.96–0.99
17	23 ± 4	167 ± 16	7.6 ± 1.8	1	0.96–0.99
18	4.2 ± 1.2	125 ± 18	33 ± 7	1	0.96–0.98
19[*]	6.9 ± 0.2	177 ± 1	25 ± 1	2	0.96–0.99
20[*]	13 ± 2	250 ± 16	20 ± 2	1	0.96–0.99
27	31 ± 4	18 ± 1	0.58 ± 0.04	1	0.95–0.99
29	67 ± 9	136 ± 3	2.1 ± 0.3	1	0.98–0.99

Results are average ± standard deviation ($n = 3$).
[a] Results for compound **2** are from reference [24].
[*] At high substrate concentrations, there was inhibition of Nterm-phos activity.

for Nterm-phos activity [7], suggesting that classic assay conditions for LPA hydrolysis resulted in total inhibition of sEH phosphatase activity.

Thus, to test if the sEH phosphatase plays a significant role in LPA hydrolysis in tissue, we measured the hydrolysis of **16** in Swiss-Webster mice tissue in the presence and absence of Triton X-100. As shown in Table 3, in the absence of Triton X-100 and presence of Mg^{2+}, there is significant LPA hydrolysis activity in the soluble fraction; around 60% of the total LPA activity in the liver and 20% in the lungs. In the presence of Triton X100, the soluble activity is reduced by more than 90%, while one third of the membrane LPA activity remained. Using a specific substrate (*t*-DPPO [30]), we estimated the amount of sEH in the tissue extract (Table 3). There is a very good correlation ($r > 0.9$) between *t*-DPPO activity and LPA hydrolysis activity in absence of Triton X-100, which disappears ($r < 0.2$) when the detergent is present. Put together, these results suggest that sEH has a significant if not major role in intracellular LPA hydrolysis.

To confirm these results, we measured LPA hydrolysis activity in wild-type and sEH-null C57BL6 mice tissues [14]. We found that there is 99% less LPA hydrolysis activity in the soluble fraction of livers from sEH-null mice (0.021 ± 0.007 nmol min^{-1} mg^{-1}; $n = 6$) than in livers of wild type mice (3.10 ± 0.7 nmol min^{-1} mg^{-1}; $n = 6$). Furthermore, we also found a 38% reduction in LPA hydrolysis activity in the soluble fraction of lungs from sEH-null mice (2.4 ± 0.5 nmol min^{-1} mg^{-1}; $n = 6$) than in lungs of wild type mice (3.9 ± 0.7 nmol min^{-1} mg^{-1}; $n = 6$).

4. Discussion

Our results support that sEH phosphatase activity prefers lipid phosphates (Table 1). Interestingly, while LPAs (**16**–**20**) inhibited significantly Nterm-phos, the corresponding di-phosphoesters (**22**–**25**) have no selective effect on this enzyme, confirming that sEH phosphatase activity prefers mono-phosphate esters [24]. The measurement of the kinetic parameters (Table 2) showed that the LPAs are the best natural substrates for Nterm-phos found so far. Interestingly, LPA hydrolysis has been reported solely for membrane bound enzymes: the lipins and lipid phosphate phosphatases [34,35]. Compared to kinetic constants published for these later enzymes, sEH hydrolyzes LPAs *in vitro* one order of magnitude faster. Because the environment in tissues differ widely from *in vitro* conditions, it is important to estimate the role on an enzyme in tissues. LPA hydrolysis activity has been reported largely in cellular membrane fractions [32], where the lipins and lipid phosphate phosphatases are localized [34,35]. We showed herein that the absence or very low level of cytosolic LPA hydrolysis reported in these studies and others is caused by inappropriate assay conditions (Triton X-100) that resulted in the inactivation of Nterm-phos [33]. We showed herein than in the absence of Triton X-100 there is a significant amount of soluble LPA hydrolysis activity in tissues (20–60% of the total activity). Furthermore, sEH represents the majority of this soluble LPA activity.

By binding to various nuclear receptors, LPAs regulate cell survival, apoptosis, motility, shape, differentiation, gene transcription, and malignant transformation [32,36]. The pharmacological regulation of LPA levels is a novel approach for the treatment of several cancers, but also inflammation and atherosclerosis leading causes of cardiovascular diseases [36]. The action of sEH on LPA could explain the observed effects of Nterm-phos on cell growth [16,18]. In addition, LPA inhibits pre-adipocyte differentiation, thus

Table 3
Effect of Triton X-100 on LPA hydrolysis activity in Swiss-Webster mice liver and lungs ($n = 6$).

	Liver		Lung	
	Soluble fraction	Membranes	Soluble fraction	Membranes
1-Myristoyl-2-hydroxy-3-glycerophosphate hydrolysis				
No Triton X-100				
Specific activity (nmol min^{-1} mg^{-1})	0.98 ± 0.20	0.61 ± 0.07	0.26 ± 0.07	1.23 ± 0.17
Total activity (nmol min^{-1} g_{tissue}^{-1})	71 ± 22	48 ± 21	10 ± 2	44 ± 7
With 5 mM Triton X-100				
Specific activity (nmol min^{-1} mg^{-1})	0.04 ± 0.02	0.19 ± 0.10	0.002 ± 0.004	0.39 ± 0.08
Total activity (nmol min^{-1} g_{tissue}^{-1})	3 ± 2	13 ± 5	0.04 ± 0.11	14 ± 3
***t*-DPPO hydrolysis**				
Specific activity (nmol min^{-1} mg^{-1})	34 ± 4	16 ± 2	2.1 ± 0.8	0.18 ± 0.04
Total activity (nmol min^{-1} g_{tissue}^{-1})	2465 ± 610	1200 ± 400	78 ± 18	6 ± 2

limiting adipogenesis, through interaction with PPARγ [37]. This nuclear receptor plays an essential role in regulating lipid and glucose homeostasis. These facts could explain the role of Nterm-phos in lipid synthesis [14,15]. It also suggests a complementary role of Nterm-phos to Cterm-EH in the development of diabetes and cardiovascular diseases [1,2,12–14]. Finally, LPA activates TRPV1 resulting in painful conditions [38]. Interestingly, we showed that Cterm-EH inhibition has analgesic properties [6], suggesting that the phosphatase and epoxide hydrolase activities of sEH may have opposite roles in pain biology.

In conclusion, the significant cellular role of sEH on LPA hydrolysis could explain some of the biology previously associated with Nterm-phos. It also underlines possible cellular mechanism in which LPA hydrolysis could be a cellular mechanism explaining how both activities of the sEH will have either complementary (cardiovascular diseases) or antagonistic (pain perception) roles.

Disclosure statement

The authors have nothing to disclose.

Declarations of interest

The authors declare no conflicts of interest.

Acknowledgments

This work was partially funded by NIEHS Grant ES02710 and NIEHS Superfund Basic Research Program Grant P42 ES04699 and by the Schormueller Foundation Grant to NHS. BDH is a George and Judy Marcus Senior Fellow of the American Asthma Foundation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2012.02.108.

References

[1] J.W. Newman, C. Morisseau, B.D. Hammock, Epoxide hydrolases: their roles and interactions with lipid metabolism, Prog. Lipid Res. 44 (2005) 1–51.
[2] B.M. De Taeye, C. Morisseau, J. Coyle, et al., Expression and regulation of soluble epoxide hydrolase in adipose tissue, Obesity 18 (2010) 489–498.
[3] G.A. Gomez, C. Morisseau, B.D. Hammock, et al., Structure of human soluble epoxide hydrolase reveals mechanistic interferences on bifunctional catalysis in epoxide and phosphate ester hydrolysis, Biochemistry 43 (2004) 4716–4723.
[4] J.D. Imig, B.D. Hammock, Soluble epoxide hydrolase as a therapeutic target for cardiovascular diseases, Nat. Rev. Drug. Discov. 8 (2009) 794–805.
[5] A. Iyer, D.P. Fairlie, J.B. Prins, et al., Inflammatory lipid mediators in adipocyte function and obesity, Nat. Rev. Endocrinol. 6 (2010) 71–82.
[6] R.H. Ingraham, R.D. Gless, H.Y. Lo, Soluble epoxide hydrolase inhibitors and their potential for treatment of multiple pathologic conditions, Curr. Med. Chem. 18 (2011) 587–603.
[7] J.W. Newman, C. Morisseau, T.R. Harris, et al., The soluble epoxide hydrolase encoded by EPXH2 is a bifunctional enzyme with novel lipid phosphate phosphatase activity, Proc. Natl. Acad. Sci. USA 100 (2003) 1558–1563.
[8] C. Morisseau, B. Inceoglu, K. Schmelzer, et al., Naturally occurring monoepoxides of eicosapentaenoic acid and docosahexaenoic acid are bioactive antihyperalgesic lipids, J. Lipid Res. 51 (2010) 3481–3490.
[9] C. Morisseau, M.H. Goodrow, D. Dowdy, et al., Potent urea and carbamate inhibitors of soluble epoxide hydrolases, Proc. Natl. Acad. Sci. USA 96 (1999) 8849–8854.
[10] J.D. Imig, Targeting epoxides for organ damage in hypertension, J. Cardiovasc. Pharmacol. 56 (2010) 329–335.
[11] J.J. Iliff, J. Jia, J. Nelson, et al., Epoxyeicosanoid signaling in CNS function and disease, Prostaglandins Other Lipid Mediat. 91 (2010) 68–84.
[12] P. Luo, H.H. Chang, Y. Zhou, et al., Inhibition or deletion of soluble epoxide hydrolase prevents hyperglycemia, promotes insulin secretion, and reduces islet apoptosis, J. Pharmacol. Exp. Ther. 334 (2010) 430–438.
[13] A. Iyer, K. Kauter, M.A. Alam, et al., Pharmacological inhibition of soluble epoxide hydrolase ameliorates diet-induced metabolic syndrome in rats, Exp. Diabetes. Res. 2012 (2012) 758614.
[14] A. Luria, C. Morisseau, H.J. Tsai, et al., Alteration in plasma testosterone levels in male mice lacking soluble epoxide hydrolase, Am. J. Physiol. Endocrinol. Metab. 297 (2009) E375–E383.
[15] A.E. EnayetAllah, A. Luria, B. Luo, et al., Opposite regulation of cholesterol levels by the phosphatase and hydrolase domains of soluble epoxide hydrolase, J. Biol. Chem. 283 (2008) 36592–36598.
[16] A. Oguro, K. Sakamoto, S. Suzuki, et al., Contribution of hydrolase and phosphatase domains in soluble epoxide hydrolase to vascular endothelial growth factor expression and cell growth, Biol. Pharm. Bull. 32 (2009) 1962–1967.
[17] B. Keserü, E. Barbosa-Sicard, R.T. Schermuly, et al., Hypoxia-induced pulmonary hypertension: comparison of soluble epoxide hydrolase deletion vs. inhibition, Cardiovasc. Res. 85 (2010) 232–240.
[18] H.H. Hou, B.D. Hammock, K.H. Su, et al., N-terminal domain of soluble epoxide hydrolase negatively regulates the VEGF-mediated activation of endothelial nitric oxide synthase, Cardiovasc. Res. 93 (2011) 120–129.
[19] M. Fornage, E. Boerwinkle, P.A. Doris, et al., Polymorphism of the soluble epoxide hydrolase is associated with coronary artery calcification in African–American subjects, Circulation 109 (2004) 335–339.
[20] K. Ohtoshi, H. Kaneto, K. Node, et al., Association of soluble epoxide hydrolase gene polymorphism with insulin resistance in type 2 diabetic patients, Biochem. Biophys. Res. Comm. 331 (2005) 347–350.
[21] B.D. Przybyla-Zawislak, P.K. Srivastava, J. Vásquez-Matías, et al., Polymorphisms in human soluble epoxide hydrolase, Mol. Pharmacol. 64 (2003) 482–490.
[22] P.K. Srivastava, V.K. Sharma, D.S. Kalonia, et al., Polymorphisms in human soluble epoxide hydrolase: effects on enzyme activity, enzyme stability, and quaternary structure, Arch. Biochem. Biophys. 427 (2004) 164–169.
[23] C. Fava, M. Montagnana, E. Danese, et al., Homozygosity for the EPHX2 K55R polymorphism increases the long-term risk of ischemic stroke in men: a study in Swedes, Pharmacogenet. Genomics 20 (2010) 94–103.
[24] K.L. Tran, P.A. Aronov, H. Tanaka, et al., Lipid Sulfates and sulfonates are allosteric competitive inhibitors of the N-terminal phosphatase activity of the mammalian soluble epoxide hydrolase, Biochemistry 44 (2005) 12179–12187.
[25] A.E. Enayetallah, D.F. Grant, Effects of human soluble epoxide hydrolase polymorphisms on isoprenoid phosphate hydrolysis, Biochem. Biophys. Res. Commun. 341 (2006) 254–260.
[26] J.K. Beetham, T. Tian, B.D. Hammock, CDNA cloning and expression of a soluble epoxide hydrolase from human liver, Arch. Biochem. Biophys. 305 (1993) 197–201.
[27] R.N. Wixtrom, M.H. Silva, B.D. Hammock, Affinity purification of cytosolic epoxide hydrolase using derivatized epoxy-activated Sepharose gels, Anal. Biochem. 169 (1988) 71–80.
[28] P.D. Jones, N.M. Wolf, C. Morisseau, et al., Fluorescent substrates for soluble epoxide hydrolase and application to inhibition studies, Anal. Biochem. 343 (2005) 66–75.
[29] N.H. Schebb, D. Falck, H. Faber, et al., Fast method for monitoring phospholipase A2 activity by liquid chromatography–electrospray ionization mass spectrometry, J. Chromatogr. A 1216 (2009) 5249–5255.
[30] B. Borhan, T. Mebrahtu, S. Nazarian, et al., Improved radiolabeled substrates for soluble epoxide hydrolase, Anal. Biochem. 231 (1995) 188–200.
[31] A.K. Das, A.K. Hajra, Critical micellar concentrations of palmitoyl dehydroxyacetone phosphate and 1-palmitoyl-rac-glycerol 3-phosphate, J. Biol. Chem. 267 (1992) 9731.
[32] V.A. Sciorra, A.J. Morris, Roles for lipid phosphate phosphatases in regulation of cellular signaling, Biochim. Biophys. Acta 1582 (2002) 45–51.
[33] M.I. McDermott, Y.J. Sigal, J.S. Crump, et al., Enzymatic analysis of lipid phosphate phosphatases, Methods 39 (2006) 169–179.
[34] D.N. Brindley, C. Pilquil, M. Sariahmetoglu, et al., Phosphatidate degradation: phosphatidate phosphatases (lipins) and lipid phosphate phosphatases, Biochim. Biophys. Acta 1791 (2009) 956–961.
[35] G.S. Han, G.M. Carman, Characterization of the human LPIN1-encoded phosphatidate phosphatase isoforms, J. Biol. Chem. 285 (2010) 14628–14638.
[36] M.E. Lin, D.R. Herr, J. Chun, Lysophosphatidic acid (LPA) receptors: signaling properties and disease relevance, Prostaglandins Other Lipid Mediat. 91 (2010) 130–138.
[37] A.J. Morris, M. Panchatcharam, H.Y. Cheng, et al., Regulation of blood and vascular cell function by bioactive lysophospholipids, J. Thromb. Haemost. 7 (Suppl. 1) (2009) 38–43.
[38] G. Tigyi, Lipids: LPA activates TRPV1 and it hurts, Nat. Chem. Biol. 8 (2011) 22–23.