Archives of Biochemistry and Biophysics 613 (2017) 1–11

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

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

Probing the orientation of inhibitor and epoxy-eicosatrienoic acid binding in the active site of soluble epoxide hydrolase

Kin Sing Stephen Lee [a], Niel M. Henriksen [b], Connie J. Ng [a], Jun Yang [a], Weitao Jia [c], Christophe Morisseau [a], Armann Andaya [c], Michael K. Gilson [b], Bruce D. Hammock [a, *]

[a] Department of Entomology and Nematology, UCD Comprehensive Cancer Center, University of California at Davis, One Shields Avenue, Davis, CA 95616, USA
[b] Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California at San Diego, 9500 Gilman Drive, MC 0736, La Jolla, CA 92093, USA
[c] Campus Mass Spectrometry Facilities, University of California at Davis, One Shields Avenue, Davis, CA 95616, USA

ARTICLE INFO

Article history:
Received 29 August 2016
Received in revised form
26 October 2016
Accepted 27 October 2016
Available online 29 October 2016

Keywords:
Photoaffinity tag
Photolabel
Soluble epoxide hydrolase
Epoxyeicosatrienoic acid
Computational simulation
Peptide sequencing

ABSTRACT

Soluble epoxide hydrolase (sEH) is an important therapeutic target of many diseases, such as chronic obstructive pulmonary disease (COPD) and diabetic neuropathic pain. It acts by hydrolyzing and thus regulating specific bioactive long chain polyunsaturated fatty acid epoxides (lcPUFA), like epoxyeicosatrienoic acids (EETs). To better predict which epoxides could be hydrolyzed by sEH, one needs to dissect the important factors and structural requirements that govern the binding of the substrates to sEH. This knowledge allows further exploration of the physiological role played by sEH. Unfortunately, a crystal structure of sEH with a substrate bound has not yet been reported. In this report, new photoaffinity mimics of a sEH inhibitor and EET regioisomers were prepared and used in combination with peptide sequencing and computational modeling, to identify the binding orientation of different regioisomers and enantiomers of EETs into the catalytic cavity of sEH. Results indicate that the stereochemistry of the epoxide plays a crucial role in dictating the binding orientation of the substrate.

1. Introduction

Soluble epoxide hydrolase (sEH, EC 3.3.2.10) degrades saturated and long-chain polyunsaturated fatty acid (lcPUFA) epoxides to the corresponding 1,2-diols [1]. The lcPUFA epoxides from both omega-3 and omega-6 lcPUFAs play an important role in human physiology and health. Studies have shown that lcPUFA epoxides are anti-inflammatory, anti-hypertensive, organ protective and analgesic [2–9]. Therefore, stabilization of lcPUFA epoxides *in vivo* through sEH inhibition is generally beneficial to human health and certain diseases, like chronic obstructive pulmonary diseases (COPD) [10,11], atrial fibrillation [12] and diabetic neuropathic pain [13–15]. To better predict whether an epoxide could be hydrolyzed by sEH, one needs to dissect the structural requirements that dictate the binding of substrate into the sEH active site. This will help us to identify other potential substrates for sEH including both endogenous and xenobiotic epoxides, which could affect its known biological roles as well as explore new roles played by sEH in human health and xenobiotic metabolism.

Over the years, numerous crystal structures of the sEH with various structurally different inhibitors have been solved [13,16–21]. Although these *holo*-structures helped us to design better inhibitors [13], these structures provide little information on how substrates bind to sEH, particularly lcPUFA epoxides. In addition, the substrate bound sEH structure has not yet been solved. To make matters more complicated, several crystal structures show that inhibitors with very similar structure bind to sEH with opposite orientations (Figure S1) [22]. These data suggest that the sEH inhibitors and substrates may bind to sEH with multiple orientations so that a crystal structure may not be able to capture all the orientations. These data also illustrate a limitation of crystallography and the need for new complementary approaches to elucidate mechanistic details of the binding of substrates and inhibitors to sEH. Therefore, in this study, computational models in combination with photoaffinity labeling experiments were conducted in

Abbreviation: TFA, trifluoroacetic acid; PAL, photoaffinity label; EET, epoxyeicosatrienoic acid; sEH, soluble epoxide hydrolase; lcPUFA, long-chain polyunsaturated fatty acid; LC/MS-MS, Liquid Chromatography with tandem mass spectrometry.

* Corresponding author.
E-mail address: bdhammock@ucdavis.edu (B.D. Hammock).

http://dx.doi.org/10.1016/j.abb.2016.10.017
0003-9861/

order to better understand how EET bind to the active site of sEH.

Computational approach has been used successfully to study the molecular details that govern the binding of substrates in the active site of the enzymes. It has also been used to identify new structural scaffold for drug design [23–31]. Molecular docking or molecular dynamics simulation have been used to identify potent inhibitors for sEH [28,32–34]. Moreover, the catalytic mechanism of sEH on modeled substrates was well studied by computational models [35–38]. Therefore, computational model provides a complementary approach for us to investigate the molecular details on how EET binds to the active site of sEH.

Besides, photoaffinity labels in combination with proteomics or radiotracers have become important tools to identify specific binding proteins of the ligand or to locate specific interactions or the orientation of the ligand in the target protein [39–42]. In this report, photolabel mimics of sEH inhibitors (mimic **7**) and endogenous substrates, epoxy-eicosatrienoic acids (EETs) were prepared (mimic **16** and mimic **17**) (Fig. 1). These mimics, in combination with peptide sequencing and computational modeling, were used 1) to test whether the inhibitor in general has several binding orientations in the sEH binding pocket, 2) to identify the binding orientation of different regioisomers of EETs in sEH binding pocket, 3) to test if carboxylate or other structural features play an important role in binding to sEH and 4) to test the hypothesis that the binding of the potent transition state mimic to sEH results a different arrangement of active site residues as compared to the binding of the substrate to sEH. In addition, the stable photolabel mimic of a tight binding sEH inhibitor allows us to optimize the conditions for photolabeling sEH with the photolabel of the substrate.

2. Materials and methods

Experimental procedures for the syntheses of the compounds used in this manuscript are described in detail in supporting information.

2.1. Experimental for biochemistry

2.1.1. Enzyme preparation

2.1.1.1. Human recombinant sEH. The purification and expression of human soluble epoxide hydrolase (sEH) was described previously followed a published procedure [43]. Briefly, the human sEH was expressed in a baculovirus system in high yield. The human sEH in cell lysate was then purified by affinity chromatography and yielded a fraction with high specific activity and apparent homogeneity on SDS-PAGE (>95%) (Figure S16). The enzyme was then quickly frozen in liquid nitrogen and stored at -78 °C. The enzyme was thawed slowly on ice before use.

2.1.1.2. Bacterial cytochrome P450 (BM3-F87V). In order to synthesized the regio- and stereo-specific 14*S*,15*R*-EET, a bacterial cytochrome P450 mutant (BM3-F87V) was used. The expression and purification of BM3-F87V was based on a published procedure [44]. Briefly, the plasmid containing bacterial P450 BM3-F87V was a generous gift from Prof. Frances A. Walker at the University of Arizona and was transformed to *E. coli* strain DH5α competent cells (Invitrogen™) according to the manufacture's protocol. The transformed cells were used for protein expression. A single colony of DH5α containing the plasmid of BM3-F87V was inoculated into 5 mL of LB/Ampiclin (100 μg/mL). The cell culture was incubated at 37 °C and shaken at 220 rpm for 12 h. The cell culture (5 mL) was then inoculated into 1.5 L of LB/Ampicilin (100 μg/mL). The larger cell culture was then incubated at 37 °C and shaken at 220 rpm for 8 h until the OD_{600} reached around 0.8 to 1.0. The cell culture was then further incubated at 30 °C and shaken at 220 rpm for an additional 16 h.

Cells were harvested at 4 °C by centrifugation for 30 min at 7000 rpm. The supernatant was discarded and the lysate buffer (100 mM Tris-HCl, 1 mM 1,14-dithiothreitol, 0.1 mM ethylenediaminetetraacetic acid, 0.1 mM PMSF, 40 mL) was added and the cells were resuspended in the lysate buffer at 4 °C. The cells were then frozen at -78 °C for 24 h. The cells were warmed up slowly in an ice bath. Once the cells were thawed, the cells were lyzed by sonication (Fisher Scientific Sonic Dismembrator Model 100, power 10, 60s on followed by 60s off for 5 times). The cell lysate was then centrifuged at 20,000 g for 30 min at 4 °C and the supernatant was transferred to another clean centrifuge tube and further centrifuged at 20,000 g for 30 min at 4 °C. The supernatant was then loaded into a column (2.5 × 20 cm) packed with anion-exchange resin (GE Healthcare Life Sciences, Q Sepharose Fast Flow). The column was washed with 5 column volumes of wash buffer (100 mM Tris-HCl, pH 7.8) followed by 5 column volumes of elution buffer (100 mM Tris-HCl, 175 mM NaCl, pH 7.8) and ended with 10 column volumes of cleaning buffer (100 mM Tris-HCl, 340 mM NaCl, pH 7.8). The red fractions were collected. The

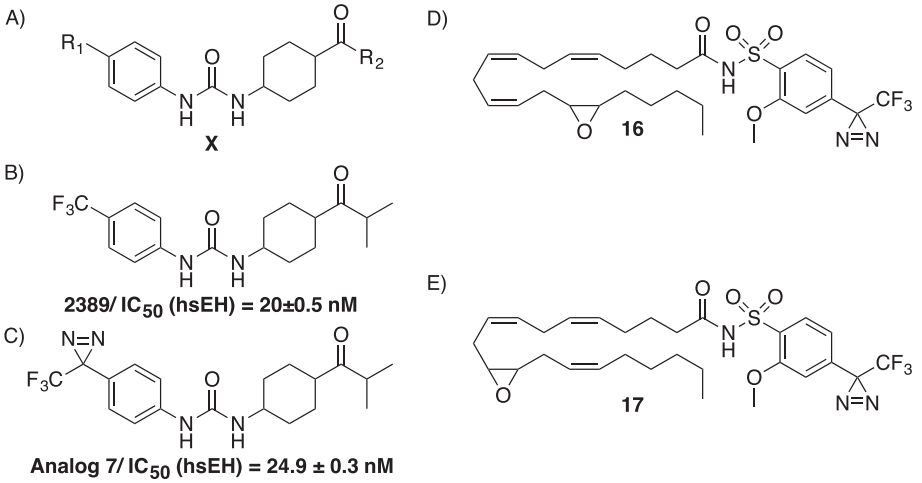

Fig. 1. A) The general scaffold of one of the leading series of sEH inhibitors; **B)** Structure of UC2389; **C)** The photoaffinity analog of UC2389 (**7**); **D)** The 14,15-EET-PAL (**16**) and; **E)** The 11,12-EET-PAL (**17**).