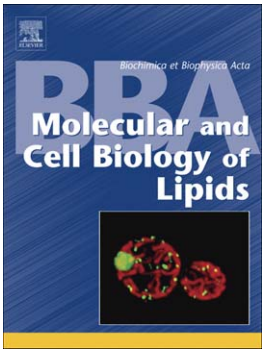

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PII: S1388-1981(17)30146-4
DOI: doi:10.1016/j.bbalip.2017.07.015
Reference: BBAMCB 58188

To appear in: *BBA - Molecular and Cell Biology of Lipids*

Received date: 6 February 2017
Revised date: 16 June 2017
Accepted date: 29 July 2017

Please cite this article as: Yana Y. Toporkova, Svetlana S. Gorina, Fakhima K. Mukhitova, Mats Hamberg, Tatyana M. Ilyina, Lucia S. Mukhtarova, Alexander N. Grechkin, Identification of CYP443D1 (CYP74 clan) of *Nematostella vectensis* as a first cnidarian epoxyalcohol synthase and insights into its catalytic mechanism, *BBA - Molecular and Cell Biology of Lipids* (2017), doi:10.1016/j.bbalip.2017.07.015

Identification of CYP443D1 (CYP74 clan) of *Nematostella vectensis* as a first cnidarian epoxyalcohol synthase and insights into its catalytic mechanism

Yana Y. Toporkova[a], Svetlana S. Gorina[a], Fakhima K. Mukhitova[a], Mats Hamberg[b], Tatyana M. Ilyina[a], Lucia S. Mukhtarova[a], Alexander N. Grechkin[a,*]

[a]Kazan Institute of Biochemistry and Biophysics, Russian Academy of Sciences, P.O. Box 30, Kazan, 420111 Russia

[b]Division of Physiological Chemistry II, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, S-17177 Stockholm, Sweden

Abstract

The CYP74 clan enzymes are responsible for the biosynthesis of numerous bioactive oxylipins in higher plants, some Proteobacteria, brown and green algae, and Metazoa. A novel putative CYP74 clan gene *CYP443D1* of the starlet sea anemone (*Nematostella vectensis*, Cnidaria) has been cloned, and the properties of the corresponding recombinant protein have been studied in the present work. The recombinant CYP443D1 was incubated with the 9- and 13-hydroperoxides of linoleic and α-linolenic acids (9-HPOD, 13-HPOD, 9-HPOT, and 13-HPOT, respectively), as well as with the 9-hydroperoxide of γ-linolenic acid (γ-9-HPOT) and 15-hydroperoxide of eicosapentaenoic acid (15-HPEPE). The enzyme was active towards all C_{18}-hydroperoxides with some preference to 9-HPOD. In contrast, 15-HPEPE was a poor substrate The CYP443D1 specifically converted 9-HPOD into the oxiranyl carbinol **1**, (9*S*,10*R*,11*S*,12*Z*)-9,10-epoxy-11-hydroxy-12-octadecenoic acid. Both ^{18}O atoms from [$^{18}O_2$-hydroperoxy]9-HPOD were virtually quantitatively incorporated into product **1**. Thus, the CYP443D1 exhibited epoxyalcohol synthase (EAS) activity. The ^{18}O labelling data demonstrated that the reaction mechanism included three sequential steps: (1) hydroperoxyl homolysis, (2) oxy radical rearrangement into epoxyallylic radical, (3) hydroxyl rebound, resulting in oxiranyl carbinol formation. The 9-HPOT and γ-9-HPOT were also specifically converted into the oxiranyl carbinols, 15,16- and 6,7-dehydro analogues of compound **1**, respectively. The 13-HPOD was converted into *erythro*- and *threo*-isomers of oxiranyl carbinol, as well as oxiranyl vinyl carbinols. The obtained results allow