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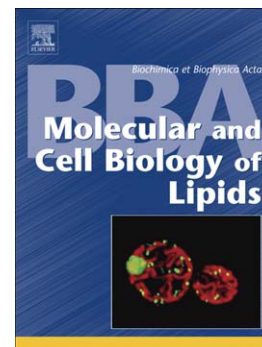
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Identification of CYP443D1 (CYP74 clan) of *Nematostella vectensis* as a first cnidarian epoxyalcohol synthase and insights into its catalytic mechanism

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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 C₁₈-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**, (9S,10R,11S,12Z)-9,10-epoxy-11-hydroxy-12-octadecenoic acid. Both ¹⁸O atoms from [¹⁸O₂-hydroperoxy]9-HPOD were virtually quantitatively incorporated into product **1**. Thus, the CYP443D1 exhibited epoxyalcohol synthase (EAS) activity. The ¹⁸O 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

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