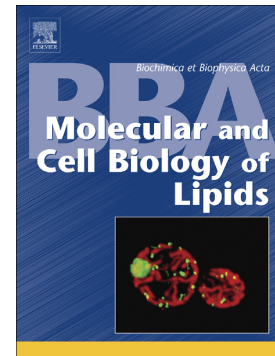


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Double function hydroperoxide lyases / epoxyalcohol synthases (CYP74C) of higher plants: identification and conversion into allene oxide synthases by site-directed mutagenesis

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**DOUBLE FUNCTION HYDROPEROXIDE LYASES / EPOXYALCOHOL SYNTHASES
(CYP74C) OF HIGHER PLANTS: IDENTIFICATION AND CONVERSION INTO
ALLENE OXIDE SYNTHASES BY SITE-DIRECTED MUTAGENESIS**

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Abstract

The CYP74C subfamily of fatty acid hydroperoxide transforming enzymes includes hydroperoxide lyases (HPLs) and allene oxide synthases (AOSs). This work reports a new facet of the putative CYP74C HPLs. Initially, we found that the recombinant CYP74C13_MT (*Medicago truncatula*) behaved predominantly as the epoxyalcohol synthase (EAS) towards the 9(*S*)-hydroperoxide of linoleic acid. At the same time, the CYP74C13_MT mostly possessed the HPL activity towards the 13(*S*)-hydroperoxides of linoleic and α -linolenic acids. To verify whether this dualistic behaviour of CYP74C13_MT is occasional or typical, we also examined five similar putative HPLs (CYP74C). These were CYP74C4_ST (*Solanum tuberosum*), CYP74C2 (*Cucumis melo*), CYP74C1_CS and CYP74C31 (both of *Cucumis sativus*), and CYP74C13_GM (*Glycine max*). All tested enzymes behaved predominantly as EAS toward 9-hydroperoxide of linoleic acid. Oxiranyl carbinols such as (9*S*,10*S*,11*S*,12*Z*)-9,10-epoxy-11-hydroxy-12-octadecenoic acids were the major EAS products. Besides, the CYP74C31 possessed an additional minor 9-AOS activity. The mutant forms of CYP74C13_MT, CYP74C1_CS, and CYP74C31 with substitutions at the catalytically essential domains, namely the “hydroperoxide-binding domain” (I-helix), or the SRS-1 domain near the N-terminus, showed strong AOS activity. These HPLs to AOSs conversions were observed for the first time. Until now a large part of CYP74C enzymes has been considered

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