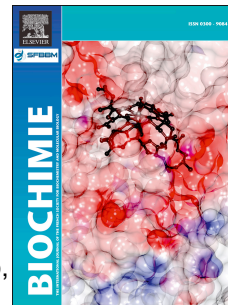


Accepted Manuscript

Two *Eucommia* farnesyl diphosphate synthases exhibit distinct enzymatic properties leading to end product preferences

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PII: S0300-9084(16)30256-5

DOI: [10.1016/j.biochi.2017.05.001](https://doi.org/10.1016/j.biochi.2017.05.001)

Reference: BIOCHI 5195

To appear in: *Biochimie*

Received Date: 21 October 2016

Revised Date: 22 April 2017

Accepted Date: 1 May 2017

Please cite this article as: H. Kajiura, N. Suzuki, Y. Tokumoto, T. Yoshizawa, S. Takeno, K. Fujiyama, Y. Kaneko, H. Matsumura, Y. Nakazawa, Two *Eucommia* farnesyl diphosphate synthases exhibit distinct enzymatic properties leading to end product preferences, *Biochimie* (2017), doi: 10.1016/j.biochi.2017.05.001.

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Abstract

Farnesyl diphosphate synthase (FPS) is an essential enzyme in the biosynthesis of prenyl precursors for the production of primary and secondary metabolites, including sterols, dolichols, carotenoids and ubiquinones, and for the modification of proteins. Here we identified and characterized two FPSs (EuFPS1 and EuFPS2) from the plant *Eucommia ulmoides*. The EuFPSs had seven highly conserved prenyltransferase-specific domains that are critical for activity. Complementation and biochemical analyses using bacterially produced recombinant EuFPS isoforms showed that the EuFPSs had FPP synthesis activities both *in vivo* and *in vitro*. In addition to the typical reaction mechanisms of FPS, EuFPSs utilized farnesyl diphosphate (FPP) as an allylic substrate and participated in further elongation of the isoprenyl chain, resulting in the synthesis of geranylgeranyl diphosphate. However, despite the high amino acid similarities between the two EuFPS isozymes, their specific activities, substrate preferences, and final reaction products were different. The use of dimethylallyl diphosphate (DMAPP) as an allylic substrate highlighted the differences between the two enzymes: depending on the pH, the metal ion cofactor, and the cofactor concentration, EuFPS2 accumulated geranyl diphosphate as an intermediate product at a constant rate, whereas EuFPS1 synthesized little geranyl diphosphate. The reaction kinetics of the EuFPSs demonstrated that isopentenyl diphosphate and DMAPP were used both as substrates and as inhibitors of EuFPS activity. Taken together, the results indicate that the biosynthesis of FPP is highly regulated by various factors indispensable for EuFPS reactions in plants.

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