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Structure and Mutation of Deoxypodophyllotoxin Synthase (DPS), a C-C Bond Forming Enzyme

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PURPOSE OF THE ABSTRACT

Deoxypodophyllotoxin synthase (DPS) is a member of the 2-oxoglutarate dependent dioxygenase (2-ODD) superfamily and catalyses a ring closing, C-C bond forming reaction. The product of this reaction, deoxypodophyllotoxin, is a polyphenol lignin ultimately derived from tyrosine via the phenylpropanoid pathway. The enzyme was discovered in 2015 during an investigation into the biosynthesis of podophyllotoxin in Podophyllum hexandrum (mayapple). (Scheme 1)[1]. Deoxypodophyllotoxin is the natural product precursor to several topoisomerase II inhibitors, which are on the World Health Organisation (WHO)'s list of essential medicines. Currently it is necessary to isolate this precursor directly from mayapple, which is slow-growing and has a limited environmental range, making it less than ideal as a source for the large-scale synthesis of topoisomerase II inhibitors[2].

Previous work has focused on developing synthetic biology platforms for the production of podophyllotoxin through heterologous gene expression, including DPS[2, 3, 4]. It has also been shown that DPS can accept a range of substrates, indicating it has potential in biocatalytic processes for the formation of diverse polycyclic aryllignans[5, 6, 7]. Here we present the structure of DPS, solved using X-ray crystallography to a resolution of 1.41 Å. The crystals were obtained in the space group P21 with a single monomer in the asymmetric unit displaying the squashed ß-barrel fold common to the 2-ODD superfamily. The open end of this supports the active site with the iron centre coordinated by the ?facial triad? of His184, D186 and H239. The structure was used to inform a mutational analysis of DPS, which suggests a role for a D224-K187 salt bridge in maintaining substrate interactions and a catalytic role for H165, perhaps as the base for the proton abstraction at the final rearomatisation step. This work improves our understanding of specific residues? contributions to the DPS mechanism and can inform future engineering of the enzyme mechanism and substrate scope for the development of a versatile biocatalyst.

FIGURES

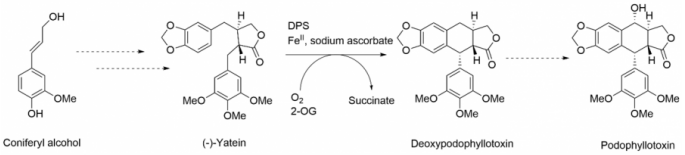

FIGURE 1

Figure 1:
The biosynthetic route to podophyllotoxin in mayapple, highlighting the penultimate step that is catalysed by DPS

FIGURE 2

KEYWORDS

oxygenase | ring-closing | deoxypodophyllotoxin | iron-dependent

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