

On substrate binding cavity of hyoscyamine 6 β -hydroxylase from devil's trumpet

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Hyoscyamine 6 β -hydroxylase (H6H) is a bifunctional enzyme that catalyzes two final steps in the scopolamine biosynthesis pathway in the *Solanaceae* family [1]. It performs hydroxylation of (2'S)-hyoscyamine at the C6 position of the tropane moiety, which yields (6S,2'S)-6 β -hydroxyhyoscyamine, and subsequent dehydrogenation of (6S,2'S)-6 β -hydroxyhyoscyamine into (2'S)-scopolamine with formation of an epoxide (**Figure 1**). However, it was recently shown that H6H can also catalyze production of (6R, 2'S)-6 β -hydroxyhyoscyamine from (2'S)-hyoscyamine at small scale [2].

H6H belongs to the family of non-heme 2-oxoglutarate/Fe(II)-dependent dioxygenases that share conserved double-stranded β -helix motif, so-called jelly-roll fold, composed of eight antiparallel β -strands. Here, we present crystal structures of H6H from *Datura metel* and its truncated version in complexes with 2-oxoglutarate, hyoscyamine and 6 β -hydroxyhyoscyamine [3]. Through analysis of the substrate binding pocket, we point out crucial residues in hyoscyamine binding and explain results of previous studies on the substrate preference of H6H.

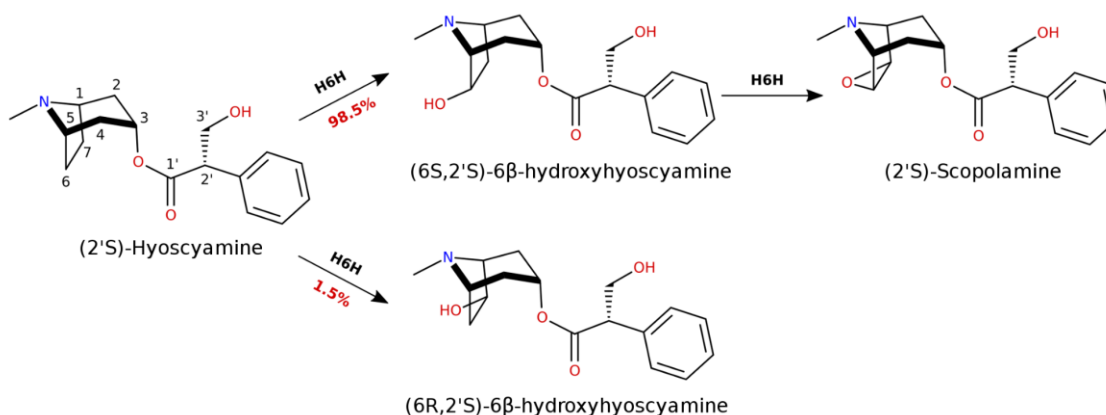


Figure 1. Two final steps in the biosynthesis of scopolamine - both catalyzed by H6H. MarvinSketch was used to draw structures and reactions [4].

[1] Hashimoto T, Yamada Y. *Plant Physiol.* 1986;81(2):619–625.

[2] Pan J, Wenger ES, Matthews ML, et al. *J Am Chem Soc.* 2019;141(38):15153–15165.

[3] Kluza A, Wojdyla Z, Mrugala B, et al. *Dalton Trans.* 2020 Apr 7;49(14):4454–4469.

[4] MarvinSketch version 18.20, ChemAxon, 2018.

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