ABSTRACT

PROBING THE CHEMISTRY IN THE ACTIVE SITE OF P4H

Prolyl 4-hydroxylase (P4H), a mononuclear, non-heme Fe^{II}, 2-oxoglutarate and O_2 dependent enzyme, plays an integral role in the biosynthesis of collagen. It catalyzes the post-translational hydroxylation of proline residues that leads to the stabilization of the collagen triple helix. Mononuclear, non-heme Fe^{II}, 2-oxoglutarate and O_2 dependent enzymes are known to catalyze a wide variety of reactions including oxidative aromatic ring cleavage, cis-dihydroxylation of double bonds, and the hydroxylation of aliphatic C-H bonds. Non-heme Fe^{II} enzymes which carry out oxidative halogenation have recently been discovered. Halogenase SyrB2 and hydroxylase P4H are related enzymes, both 2-oxoglutarate and oxygen dependent. It is thought that both the halogenation and hydroxylation reactions proceed via a similar mechanism, each performing an extremely difficult reaction, abstracting a hydrogen atom from an aliphatic carbon, to allow the formation of a halogenated or hydroxylated product. Here we report that replacing the aspartate residue in the active site of P4H with alanine, so that the active site now mimics that of the halogenase SyrB2 was not successful in converting P4H to do halogenation chemistry. In addition, switching the relative positions of residues involved in the active site in wild-type P4H results in the loss of hydroxylase activity and no gain in halogenase activity when critical residues in P4H were substituted to mimic that of a halogenase.

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