

Streptomyces wadayamensis MppP is a PLP-Dependent Oxidase, Not an Oxygenase

Lanlan Han†Nemanja Vuksanovic†Sarah A. Oehm†Tyler G. Fenske†Alan W. Schwabacher†Nicholas R. Silvaggi*†

† Department of Chemistry and Biochemistry, University of Wisconsin–Milwaukee, , , United States

Biochemistry, Article ASAP

DOI: 10.1021/acs.biochem.8b00130

Publication Date (Web): February 23, 2018

Copyright © 2018 American Chemical Society

*Postal address: 3210 North Cramer Street, Milwaukee, WI 53211, USA.

Tel.: 414-229-2647. E-mail: silvaggi@uwm.edu.

Note: Sarah is Deb Immel's daughter

Abstract

The PLP-dependent l-arginine hydroxylase/deaminase MppP from *Streptomyces wadayamensis* (SwMppP) is involved in the biosynthesis of l-enduracididine, a nonproteinogenic amino acid found in several nonribosomally produced peptide antibiotics. SwMppP uses only PLP and molecular oxygen to catalyze a 4-electron oxidation of l-arginine to form a mixture of 2-oxo-4(S)-hydroxy-5-guanidinovaleric acid and 2-oxo-5-guanidinovaleric acid. Steady-state kinetics analysis in the presence and absence of catalase shows that one molecule of peroxide is formed for every molecule of dioxygen consumed in the reaction. Moreover, for each molecule of 2-oxo-4(S)-hydroxy-5-guanidinovaleric acid produced, two molecules of dioxygen are consumed, suggesting that both the 4-hydroxy and 2-keto groups are derived from water. This was confirmed by running the reactions using either $[18]O_2$ or $H_2[18]O$ and analyzing the products by ESI-MS. Incorporation of $[18]O$ was only observed when the reaction was performed in $H_2[18]O$. Crystal structures of SwMppP with l-arginine, 2-oxo-4(S)-hydroxy-5-guanidinovaleric acid, or 2-oxo-5-guanidinovaleric acid bound were determined at resolutions of 2.2, 1.9, and 1.8 Å, respectively. The structural data show that the N-terminal portion of the protein is disordered unless substrate or product is bound in the active site, in which case it forms a well-ordered helix that covers the catalytic center. This observation suggested that the N-terminal helix may have a role in substrate binding and/or catalysis. Our structural and kinetic characterizations of N-terminal variants show that the N-terminus is critical for catalysis. In light of this new information, we have refined our

previously proposed mechanism of the SwMppP-catalyzed oxidation of l-arginine.

Ref: <https://pubs.acs.org/doi/10.1021/acs.biochem.8b00130>