Structure/Function Analysis of Pseudomonas putida Nicotine Oxidoreductase

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Several soil bacteria have adapted to using nicotine as a growth substrate and developed biochemical strategies for using it as a primary source of carbon and nitrogen. In *Pseudomonas putida*, the flavoenzyme nicotine oxidoreductase (NicA2) catalyzes the first committed step of nicotine degradation via the amino-ketone pathway forming N-methyl-myosmine, followed by non-enzymatic hydrolysis to pseudooxynicotine. Taking advantage of its unique evolutionary adaptation, we plan to refine NicA2's inherent catalytic function to develop novel therapeutics for smoking cessation and tools for tobacco waste bioremediation.

Recently, our X-ray crystallographic structure together with bioinformatics analysis of NicA2 (*Biochemistry* 2016, 55, 6595−98) established this enzyme as a member of the monoamine oxidase family. Structural analysis of NicA2 depicted an overall conserved amine oxidase fold along with a conserved FAD binding domain. The substrate-binding domain, however, contained unusual differences from canonical monoamine oxidases with a unique composition of the aromatic cage (W427 and N462) and a predominantly hydrophobic active site environment.

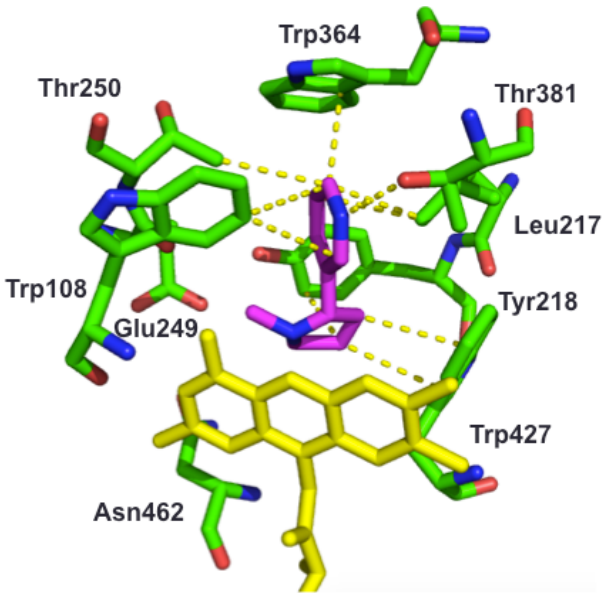

Figure 1 Stick model depicting the active site of NicA2 with the FAD cofactor and L-nicotine bound. Residues are shown in green, the isoalloxazine ring of FAD is in yellow and L-nicotine in magenta. Hydrogen bonds and hydrophobic interactions are depicted as dashed yellow lines.

Herein, we report the first structure of NicA2 in complex with the substrate, L-nicotine, refined to 2.6 Å resolution. The amine substrate is oriented proximal to the isoalloxazine ring of flavin, with the pyrrolidone ring nitrogen puckered towards the C4a of FAD, consistent with oxidation of the C-N bond (Figure 1). This orientation is largely driven by hydrophobic interactions with W108, T250, W364, L217, Y218 and W427, with the exception of a hydrogen bond formed between the pyridine nitrogen and the hydroxyl group of the side chain of T381. The hydrophobic character of the active site is consistent with binding of a deprotonated form of L-nicotine and subsequent release of the cationic N-methyl-myosmine product. Further studies probe the functional role of the aromatic cage and its role in catalysis. These studies focus on mutation of the residues W427 and N462 to resemble those of other amine oxidase family members and investigate the role of N462 in substrate binding and specificity.