I. Introduction

The concept of antibiotic resistance, the ability of an organism to grow in the presence of an antibiotic (Science Daily, 2016), is one of the primary concerns that accompanies antibiotic use. When such a bacterium carries several antibiotic resistance genes, it is referred to as multi-drug resistant, or a "superbug," such as methicillin-resistant Staphylococcus aureus (MRSA). Killing superbugs requires new and improved antibiotics not susceptible to current resistance mechanisms. Mannopeptimycin, a naturally-produced antibiotic, is active against MRSA, but development of the natural compound into a viable antibiotic has been hampered by the fact that one of the building blocks, the non-proteinogenic amino acid L-enduracididine, is not available and is difficult to make synthetically. Knowing how L-End is synthesized will aid in the development of an inexpensive route to large quantities of L-End.

II. The L-enduracididine Synthesis Pathway

The conversion of the amino acid, L-arginine (L-Arg), to the non-traditional amino acid, L-enduracididine (L-End), is achieved in a 3-step biosynthesis pathway. The first step (1) in the pathway, accomplished with the enzyme MppP, adds an oxygen atom to the L-Arg substrate, and replaces the α-amino group with a ketone to create a 4-hydroxy-2-ketoarginine (4HKA). The 4HKA is used in the second step (2), the cyclization of 4HKA by MppR to give the ketone form of End, 2-ketoenduracididine (2KE). The last step (3) is achieved by the enzyme, MppQ, which transfers an amino between 2KE and alanine or glycine to give L-End, which is then incorporated into antibiotics.

III. MppP Structural Components

There are several residues of importance in the active site of the MppP protein:
- Ser91, Asn160, Asp188, and Ser190 (purple) hold the PLP
- Thr12 and Glu15 (fuchsia) contact and cover the active site
- Asp227, Arg352, and Asp27 (gold) hold the L-Arg substrate (B, light cyan) in the active site
- PLP cofactor (silver) hydroxylates Arg producing 4HKA (C, light sky blue)
- Lys221 (lime green) is involved in the catalytic process
- Oxygen atoms are red, nitrogen atoms are blue, phosphorus atoms are orange

IV. Enzymatic Action of MppP

MppP follows typical Michaelis-Menten kinetics where the enzyme and substrate interact at the active site to form the enzyme-substrate complex. After reacting, the substrate has been converted to product and leaves the enzyme intact (Figure 4a). Through the PLP cofactor, MppP converts L-Arg and O2 to 4HKA.
- PLP bonds to the α-amino group of L-Arg
- Produces a quinonoid intermediate
- In presence of molecular oxygen, L-Arg is hydroxylated
- Produces 2 possible products (Figure 3): 4HKA and 2-ketoarginine
- Supported by NMR data (Figure 4b)

V. Experimental Evidence for Action of MppP

Data indicate that presence of the quinonoid intermediate and oxygen is necessary for the formation of 4HKA.

VI. Conclusions

"Superbugs" have become immune to traditional antibiotics. This has caused a need to develop new antibiotics, which can be expensive to synthesize. S. wadayamensis is capable of generating L-End, an interesting component. MppP is involved in the first step in synthesizing L-End. The 4HKA product of the MppP-PLP dependent hydroxylation is used in subsequent reactions in the pathway leading to the creation of the amino acid L-End, used in some antibiotics. Easy production may help researchers to cheaply and efficiently create new antibiotics.

References