STUDY OF PVCA AND PVCB, TWO ENZYMES INVOVLED IN MAKING ISONITRILE-CONTAINING NATURAL PRODUCTS IN BACTERIAL PATHOGENS

Jing Zhu

Dr. Peter Tipton, Dissertation Supervisor

ABSTRACT

Isonitriles are versatile intermediates in organic synthesis. A variety of isonitriles are found in biological systems. Some of them are produced by bacterial pathogens and may play important roles in host-pathogen interactions. The chemical and functional diversity of these novel compounds may offer important synthetic implications and pharmaceutical potential.

PvcA and PvcB are two novel enzymes involved in isonitrile biosynthesis. Early studies identified PvcA as an isonitrile synthase and PvcB as an oxygenase. But the *in vitro* activities of these proteins remained undetermined until recently.

By heterologously expressing the PvcB proteins and synthesizing its putative substrate tyrosine isonitrile, we confirmed PvcB to be an Fe^{2+}, α-ketoglutarate dependent oxygenase, and to be a source of the diversity in isonitrile-containing metabolites. The various activities exhibited by the PvcB homologs from different pathways might result from the subtle differences of the proteins in the active sites.

PvcA is predicted to convert the amino group of tyrosine into the isonitrile functionality. By overexpressing XnPvcA in *E. coli*, we confirmed tyrosine isonitrile to be the product of the PvcA reaction. Through the *in vivo* feeding and labeling studies, we demonstrated tyrosine as one of the substrates for the PvcA enzyme. The other substrate which provides the isonitrile carbon was searched broadly and was only found in the *E. coli* cell lysate. Fractionation of the lysate identified a macromolecule, like a metabolic enzyme, and a small compound, probably phosphorylated, were both required for PvcA activity.