Activity-Based Labeling of IlvE: a PLP-Dependent Enzyme from *Mtb*

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Pyridoxal-5-phosphate (PLP)-dependent enzymes catalyze an extraordinary diversity of chemical reactions in both primary and secondary metabolic pathways. The human genome encodes more than 400 PLP-dependent enzymes while many pathogenic microorganisms contain dozens of essential PLP-dependent enzymes. A number of FDA-approved drugs are known that covalently modify PLP-dependent enzymes exemplified by vigabatrin, carbidopa, D-cycloserine, and eflornithine used to treat epilepsy, Parkinson’s, tuberculosis, and African sleeping sickness, respectively. Moreover, drug discovery efforts are ongoing for numerous PLP-dependent enzymes in oncology, infectious disease, and neuroscience. In general, many of these aforementioned drugs are characterized by substantial side effects that are incompletely understood, but likely due to inhibition of other functionally related enzymes. Activity-based protein profiling (ABPP) is a powerful technique that has become popular in the field of chemical biology for characterizing enzyme selectivity and target identification of tool compounds; however, there is remarkably no described probe for PLP-dependent enzymes, one of the most important enzyme classes in biology and human medicine. We have successfully synthesized an activity-based probe capable of covalently labeling PLP-dependent enzymes. Preliminary data using purified IlvE from *Mycobacterium tuberculosis*, a PLP-dependent aminotransferase enzyme responsible for biosynthesis of the branched-chain amino acids, shows that our probe is fully capable of labeling. The native substrate for IlvE bears no structural resemblance to this probe suggesting it will promiscuously label other PLP-dependent enzymes. We currently seek to utilize this probe to label an *E. coli* strain that overexpresses *Mtb* IlvE. We anticipate that this work will allow for the profiling of enzymes in this class and the many FDA-approved drugs that target them to determine the mechanistic basis of their toxicity and potentially identify other unknown mechanisms of action of these multi-targeting inhibitors.