The Role of Glu467 in NIS Synthetase DesD: Structure, Thermodynamics, and Kinetics.

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Pathogenetic bacteria are becoming more resistant to current antibiotics with increased exposure. A simple infection that could be easily eliminated previously becomes more difficult to get rid of with the high resistance of MRSA, anthrax, and others. Creating a new class of antibiotics will be critical for our future ability to survive infections. The long term goal of the Hoffmann lab is to utilize structure-based drug design to design a new class of antibiotics. Our target is a unique family of proteins in bacteria called NIS Synthetases, involved in siderophore biosythesis; our model enzyme is Desferrioxamine D (DesD) from Streptomyces coelicolor.

Siderophores are small molecular iron chelators that are produced by microbes and whose most notable function is to sequester iron from the host and provide this essential metal nutrient to microbes. They are synthesized from multiple different pathways, however, the NIS pathways are relatively understudied, and their siderophore products are increasingly associated with bacterial virulence. NIS pathways always have one member of a unique family of enzymes called NIS Synthetases-excellent drug targets because of their associating with virulence, unique structure, and unique chemistry. A model enzyme from this family is DesD from Streptomyces coelicolor bacterium, which catalyzes the last three bonds made in the siderophore dfoE.

The literature on DesD has indicated a qualitative broad substrate specificity due to its iterative, successive bonds to the same substrate. We will test this hypothesis with quantitative binding data using isothermal titration calorimetry, and predict a significant preference for HSC (small substrate) and dfoG (large substrate) over analogs (dfoB or cadaverine) or products (dfoE). These studies will additionally allow us to describe the differences in binding to small vs. increasingly larger substrates in a quantitative exploration of iterative activity. To ensure that binding dynamics are not upstaged by catalytic turnover, we will use catalytically inactive variant Arg306Q DesD in assay using both cofactor and substrates.

Our previously published structures of DesD agree with the literature in a very important feature: the novel ATP binding site, notable for highly strained phosphate bonds enabling hydrolysis at the beta phosphate rather than the gamma (producing pyrophosphate rather than phosphate.) To explore the critical role for magnesium coordination and phosphate structure, we will present the structure, kinetics, and binding studies of variant Glu467Ala, which has a dual catalytic and magnesium ion coordination role. The combination of data will test the hypothesis that the absence of phosphate strain permits unproductive ATP hydrolysis at high [ATP] concentrations.

This project is key to future studies mapping the binding site and testing the binding of designed inhibitors to siderophore synthesis. The successful creation of an antibiotic targeted against NIS Synthetases would represent a new drug class against some of the most pathological and virulent bacteria posing threats to the human immune system.