Towards a Structural and Atomic Mechanism for NIS Synthetase DesD: Laying the Groundwork for Structure-Based Drug Design

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Recently, the family of adenylating enzymes was expanded to include two subfamilies of siderophore synthetases. One of them, the NIS Synthetases, is an excellent target for structure-based antibiotic drug design due to its novel structure and function, and association with virulence. The adenylating NIS synthetases are understudied, however, with only four different proteins structurally characterized of a family wide enough to have three subtypes and iterative behavior. Additionally, a full complex of protein, cofactor, carboxylate and amine substrates has been elusive. Biochemical characterization too, has lagged, though three different assays have been published, their range of reported values is inconsistent.

The desferrioxamine siderophores (dfoD, G, E and B) are made by Streptomyces coelicolor (and other Streptomyces) bacteria through the desferrioxamine A, B, C and D pathway (DesABCD). DesD, a type C NIS synthetase, iteratively catalyzes the last three bonds made in this pathway to sequentially create all of the known siderophores in S. coelicolor, and end with the macrocyclized dfoE, a circular trimer of N-hydroxy, N-succinyl cadaverine. We have solved the structure of DesD in apo and ATP-bound forms, as well as explored several loss-of-function variants in pursuit of a mechanistic hypothesis. Additionally, a label-free, ITC-based kinetics assay has been instrumental in describing the allosteric regulation of two binding sites creating three bonds on an increasingly complex substrate. We now present binding studies to elucidate the structural mechanism of communication between sites and steps. Kinetics indicates a negative cooperativity, which would preferentially fill one catalytic site at a time. However, it is unclear whether equilibrium associations preference the larger substrates, thereby ensuring that the largest dfoE is made as quickly as possible, or the smaller building blocks, building up more substantial concentrations of the intermediate products such as dfoD and dfoG. We will present thermodynamic, kinetic and structural data to test these competing hypotheses, all of which will help guide future attempts to crystallize the complex and lay the full foundation for understanding important steps to inhibit. This will complete the groundwork needed for structure-based drug design and testing.

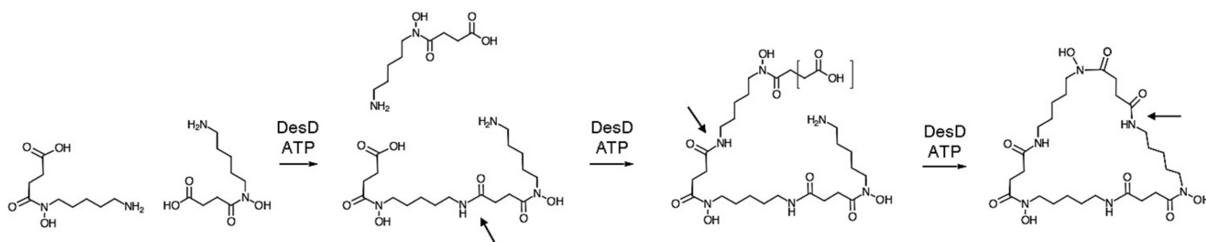

Figure 1: Scheme 1: DesD catalyzes three sequential peptide bonds (indicated with arrows) between three N-hydroxy-N-succinyl cadaverine (HSC) substrates. In step one, two small HSC molecules are joined to form an intermediate “dipeptide”, which in step 2 is connected to a third molecule of HSC to form the large tripeptide (desferrioxamine G). The final step cyclizes the tripeptide to form the product siderophore desferrioxamine E. Each bond formation event requires a stoichiometric equivalent of ATP to drive the reaction. A version of the tripeptide where the carboxyl group (in brackets) is missing is also commercially available and has been used since the 1970s to treat iron overload as deferoxamine B[3].