Structural studies and structure-guided engineering of epimerization domains from antibiotic biosynthetic pathways

Wei-Hung Chen,[1] and Steven D. Bruner[1]

1. Departments of Chemistry, University of Florida

Category: Chemical Biology and Biophysics

Nonribosomal peptide synthetases (NRPSs) are large, complex multi-domain enzymes responsible for the biosynthesis of a wide range of peptidyl natural products. Inherent to synthetase chemistry is the thioester templated mechanism that relies on protein/protein interactions and intra domain dynamics. Several questions related to structure and mechanism remain to be addressed, including the incorporation of accessory domains and inter-module interactions. The addition of further chemical modifications contributes significantly to the structural diversity of nonribosomal peptides (NRPs), adding functional diversity and *in vivo* stability. Notably, epimerization domains, embedded in NRPSs assembly lines, catalyze the L- to D- amino acid conversion. The inclusion of nonproteinogenic D-amino acids into peptide frameworks is a common and important modification for bioactive NRPs.

A unique and mechanistically interesting aspect of NRPS assembly lines is the overall structure and dynamics of the multidomain/multimodular machine. General questions related to domain orientation and the flux of Ppant-tethered intermediates through the large assembly remain largely unanswered. The issues of structure and mechanism have direct relevance to efforts to engineering NRPSs through domain manipulation and rearrangements to produce novel compounds, although studies have shown success, generally applicable approaches are not known. The structural complexity of many NRPs renders total synthesis impractical and semi-synthesis challenging. Therefore the bioengineering approaches consequently provide an alternative for substitution of difficult chemistry in the generation of modified peptides scaffold with altered bioactivity or improved physicochemical properties.

Here we present the structure of the epimerization domain/peptidyl carrier protein didomain construct from the first module of the cyclic peptide antibiotic gramicidin synthetase. Structures of the GrsA didomain PCP-E fragment (*holo* and *apo*) are determined by X-ray crystallography at 1.9 Å and 2.3 Å resolution, each representing catalytically relevant conformation of the two domains. Our work also provides a structural basis for the inter-module PCP-C interaction that is a key component of the all NRPS assembly lines. From the structures, novel details related to the interactions between catalytic residues and the Ppant prosthetic group and the interface region within linker-domain and domain-domain are apparent. Besides, the information revealed within structures provide insights into domain-domain recognition, substrate delivery during the assembly line process and guided us to carry out a structural based engineering with relaxed substrate specificity. In addition, the structures supply information into the structural organization of homologous condensation domains, canonical players in all synthetase modules.