Poster Presentation

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Towards the structure of an unusual nonribosomal peptide synthetase

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Cereulide synthetase (Ces) is a macromolecular machine that produces a toxin involved in severe and lethal food poisoning. Ces belongs to the nonribosomal peptide synthetase (NRPS) family protein, and as such, it employs an assembly line mechanism to generate its cereulide product. The assembly line for cereulide differs from the canonical NRPS pathway because it adds hydroxy acids instead of amino acids at half of the positions in the molecule. To achieve this, the megaenzyme selects alpha keto acids through specialized adenylation domains and then reduces them to hydroxy acids in embedded ketoreductase domains, before adding them to the growing depsipeptide. Several details on the structural features of this unusual NRPS remain unknown. Here we report a full biochemical characterization of this highly complex megaenzyme. We obtained the kinetic constants of each of its four adenylation domains and probed the side chain selectivity for its alpha keto acid – recognizing adenylation domains. Also, we report that the activating protein MbtH is not required for full activity of the assembly line. Finally, we show that each of the modules can be inhibited through mechanism-based inhibitors, which can restrain the conformational flexibility paving the way for structural studies by X-ray crystallography.

Keywords: nonribosomal peptide synthetases, macromolecular machines, NRPS