UNDERSTANDING AND MANIPULATING NON-TEMPLATED PEPTIDE BOND FORMATION BY MACROCYCLASE ENZYMES

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Peptide macrocycles are attractive molecules because they are drug-like, protease resistant, cell permeable, and possess a rigid structure. They have been shown to possess various biological activities and to be able to inhibit protein-protein interactions and other complex targets. Although several macrocyclases have been characterized to date, only two can catalyze the formation of cyclic peptides containing less than 9 amino acids in their core. PatGmac, from the biosynthesis of cyanobactins, is a versatile catalyst with very broad substrate specificity. It can utilize varied peptide sequences, incorporate unnatural amino acids, including substrates that are peptide "chimeras" containing triazoles, peg linkers and sugars (Figure 1A, bottom). Despite its remarkable substrate promiscuity, PatGmac is extremely slow, with turnover rates in the vicinity of once per day. In search for a more efficient macrocyclase we studied GmPOPB, a prolyl oligopeptidase from the mushroom *Galerina marginata*. GmPOPB (fast macrocyclase) participates in the biosynthesis of the toxic amanitins, catalyzing both peptide bond hydrolysis and peptide bond formation with equal efficiency (Figure 1A, top). We determined crystal structures of apoGmPOPB and GmPOPB mutants bound to a peptidase and a macrocyclase substrate unveiling a mechanism by which the enzyme controls which reaction will be catalyzed. We have also performed an extensive kinetic analysis of this enzyme in comparison to the slow PatGmac. Crucial differences exist between the fast and the slow macrocyclases. Substrate positioning plays an important role towards catalytic efficiency. For the fast macrocyclase GmPOPB there is product inhibition and the rate-limiting step for the reaction is product release. For the slow macrocyclase PatGmac product release is not rate determining for the majority of the substrates tested, and the rate-limiting step is coupled to chemistry. Guided by our kinetic studies, we have designed modified peptide substrates, which eliminate the requirement for a long peptide substrate from 25 amino acids to 13 amino acids for the fast macrocyclase. We are currently designing enzyme variants to improve the catalytic efficiency of the slow macrocyclase and to broaden the substrate scope of the fast macrocyclase. We hope our findings will result in a better, more efficient and substrate permissible macrocyclase that can be used for the biocatalytic generation of cyclic peptide libraries to be tested for biological function.

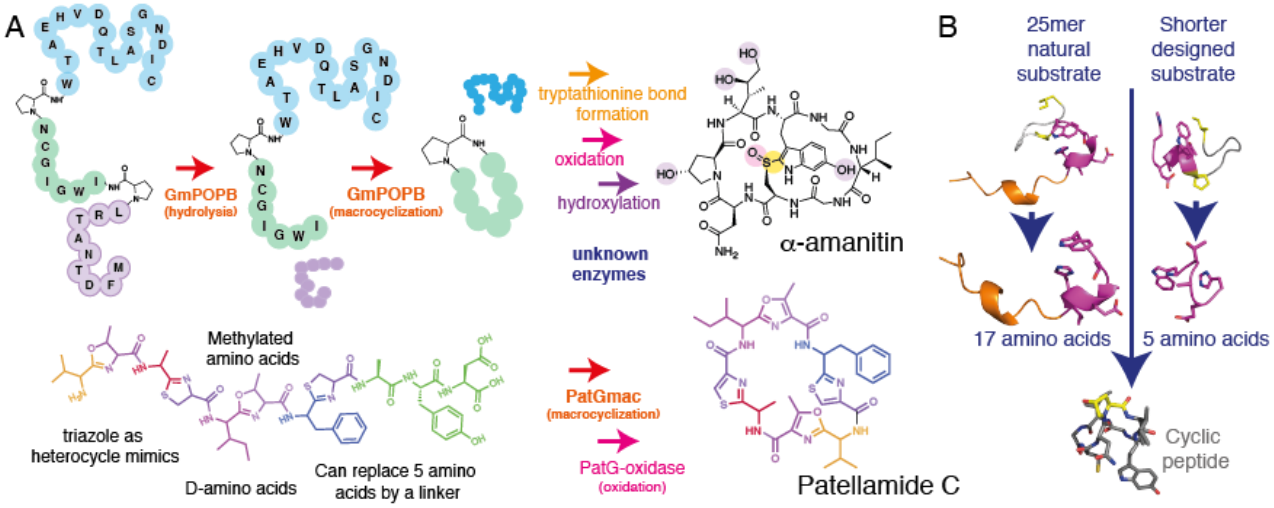

Figure 1: A) Biosynthesis of amanitins (top) and cyanobactins (abstract) and role of GmPOPB and PatGmac. B) Guided by our results we designed a modified macrocyclization substrate that allows the utilization of 13 amino acid substrates instead of the original 25, decreasing the waste of the pathway