Development of a biotechnological platform for the production of multiply backbone N-methylated peptide macrocycles

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Peptide backbone N-methylations and macrocyclization are desired properties for the development of peptide therapeutics, as these modifications can improve cell permeability, target selectivity and proteolytic stability. One famous example of a backbone N-methylated peptide macrocycle used as a therapeutic is the immunosuppressant cyclosporin A, a fungal non-ribosomal peptide natural product. Another fungal peptide natural product with structural similarity to cyclosporin A is omphalotin A. This peptide macrocycle is a ribosomally produced and post-translationally modified peptide (RiPP) that consists of 12 amino acids, 9 of which are backbone N-methylated. This compound, produced by the mushroom *Omphalotus olearius*, exhibits strong toxicity against nematodes by an unknown mechanism. In our laboratory, we investigate the biosynthetic pathway of omphalotin A as a potential biotechnological platform to produce multiply backbone N-methylated peptide macrocycles. The omphalotin A precursor protein contains a SAM-dependent α-N-methyltransferase domain, which iteratively methylates the core peptide located at the C-terminus. After complete methylation, the core peptide is cleaved off by an unidentified proteolytic activity and subsequently macrocyclized by a prolyl oligopeptidase (POP)-type enzyme. Heterologous production of omphalotin A by expression of the precursor protein and the POP-type macrocyclase has so far only been successful in yeast, but not in *Escherichia coli*. We now established the production of omphalotin A and related backbone N-methylated peptide macrocycles in *E. coli* by additional co-expression of a canonical fungal POP. In contrast to yeast, the recombinant bacteria secrete the produced omphalotin A into the culture supernatant. This heterologous production platform will enable the biosynthesis of novel backbone N-methylated peptide macrocycles with potentially promising pharmacological properties. In addition, the efficient bacterial production of omphalotin A will allow us to screen for the still unknown molecular target of omphalotin A.