In vitro Characterization of a Heterologously Expressed Non-Ribosomal Peptide Synthetase Involved in Phosphinothricin Tripeptide Biosynthesis

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Phosphinothricin tripeptide (PTT) is a widely used herbicide produced by *Streptomyces* species. Despite its use, the final steps of the PTT biosynthesis involving the peptide assembly and the rare C-P-C bond formation via P-methylation are not well understood. The unusual phosphinic acid, the active part of the peptide, is nonribosomally combined with two alanines by three PTT synthetases with PhsA previously believed to be responsible for activating and loading the unusual amino acid AcDMPt. PhsA was heterologously expressed and purified from *E. coli*, and its substrate specificity was assessed via [^{32}P]PP$_i$-ATP exchange assay revealing that PhsA activates AcPt better than AcDMPt. The 110 kDa MBP-PhsA loaded with either AcDMPt or AcPt via a thioester bond linkage was confirmed by FT-MS. These results suggest that AcPt and not AcDMPt is the natural substrate of PhsA, which could also be an indication that P-methylation takes place prior to the peptide assembly.