Abstract

Lincosamides form a small but important group of specialized microbial metabolites with antibiotic activity. The most important members of this group are celesticetin and clinically used lincomycin. Structurally, lincosamides are composed of an amino sugar and an amino acid connected by an amide bond. The amino acid precursors of both lincosamides remarkably differ. Proteinogenic L-proline is the precursor of celesticetin, while an unusual amino acid (2*S*,4*R*)-4-propyl- L-proline (PPL) is incorporated in the more efficient compound lincomycin. Surprisingly, both these precursors are recognized and activated for further biosynthetic steps by homologous adenylation domains CcbC and LmbC, respectively. The detailed description of this amino acid recognition and activation step, which is critical for the biological activity of the resulting compound, was the aim of the first part of this thesis. The site-directed mutagenesis of the LmbC substrate binding pocket and biochemical characterization of resulting mutants were employed to identify the residues crucial for the activation of PPL. Subsequently, we experimentally simulated the molecular evolution leading from L-proline-specific substrate binding pocket (like in CcbC) to the PPL-specific enzyme (LmbC). The substitution of only three amino acid residues in the substrate binding pocket was sufficient for this significant change of substrate specificity.

Once activated, the amino acid precursor is subsequently condensed with the amino sugar moiety by a unique condensation system, coupled with the metabolism of mycothiol, which acts also as a donor of the sulphur atom. The mycothiol-derived lincosamide intermediate must be further *N*-deacetylated. This reaction is a prerequisite for subsequent maturation steps, which allow the methylation of sulphur in the lincomycin biosynthesis or the attachment of salicylate in the celesticetin biosynthesis. In the second part of the thesis we elucidated the post-condensational *N*-deacetylation step and characterized previously unknown enzyme catalysing this reaction. Specifically, we showed that the *N*-deacetylation is catalysed by CcbIH/CcbQ in celesticetin biosynthesis and homologous protein pair LmbIH/LmbQ in biosynthesis of lincomycin. These are the first *N*-deacetylases belonging to a widely distributed, but poorly studied TldD/PmbA protein family, which was up to date known to comprise only endopeptidases. The uniqueness of lincosamide proteins within the TldD/PmbA protein family was also supported by the bioinformatic analysis.

Our findings concerning the key steps of lincosamide biosynthesis provided important information for understanding the molecular evolution of these unique metabolites as well as for possible *in vivo* preparation of more potent hybrid lincosamide-based compounds.

Key words: Lincosamides, adenylation domains, *N*-deacetylation, TldD/PmbA protein family